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Breeding and Seed Orchard Plan For The Development of Blister Rust Resistant White Pine for the Northern Rockies

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BREEDING AND SEED ORCHARD PLAN FOR THE DEVELOPMENT OF BLISTER RUST RESISTANT WHITE PINE FOR THE NORTHERN ROCKIES

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and
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Expanding and updating the preceding plans prepared by:

Hoff and McDonald 1978
Franc and Howe 1992, 1984

USDA Forest Service Northern Region

Inland Empire Tree Improvement Cooperative

EXECUTIVE SUMMARY

The Northern Region is very fortunate to have a head start in aggressively pursuing the goals of improving forest health in Inland Northwest ecosystems (Mahalovich in press). We also have an example of a model program in western white pine where basic research, applied technology, and funding levels have not been limiting factors to improving forest health. Estimates of western white pine in Inland forests have ranged from 40-60% of the total composition prior to the introduction of the exotic disease in the early 1900s. Recent surveys (Interior Cedar-Hemlock-White Pine Forests Symposium Proceedings 1994) have shown western white pine to make up only 4-10% of the total forest composition. Through cooperation with USFS Research and the Inland Empire Tree Improvement Cooperative, we are well under way in restoring western white pine in our Inland Northwest ecosystems. The Northern Region continues to recognize the importance of tree improvement in the context of ecosystem management.

The original document titled "Selection Methods for the Development of Blister Rust Resistant Western White Pine for the Northern Rockies" (Hoff and McDonald 1978), governed the Northern Region's Phase II or operational White Pine Tree Improvement program. The first revision of that plan, "A Plan for the Development of Blister Rust Resistant White Pine for the Northern Rockies," refined the rust resistance testing process (Franc and Howe 1992).

This revision capitalizes on the knowledge gained by advancing both horizontal and vertical resistance, which are reflected in the design of production seed orchards and advanced generation breeding strategies. This document focuses on the turning point of the western white pine breeding program as we successfully complete the first generation of improvement via plus tree selections, testing and establishment of seed orchards into the second generation of improvement. In 1994, the USFS became self sufficient in meeting our operational reforestation needs for the next two years. This trend is expected to continue as our Phase I orchards have now reached reproductive maturity and are being intensely managed via irrigation, fertilization, insect control, and supplemental mass pollination techniques.

Perhaps now is the place to interject some words of caution as we implement ecosystem management: one generation of resistance improvement is not enough. Phase I orchards are based only on known vertical resistance mechanisms, with a limited genetic base, i.e., there are 24 unrelated genotypes in each of the low (Moscow Arboretum), mid and high elevation (Lone Mountain) seed orchards, with unknown levels of horizontal resistance.

Along with restoring western white pine, we are also sensitized to risk analysis and risk ratings. Western white pine after one generation of improvement is still at risk to this exotic pest. Based on the Phase I program started in the 1950s, we can now produce seedlings that afford resistance levels in the range of 60-80%. This complex of resistance is largely based on three, single gene traits. The Northern Region is still at risk to changes (mutations) in the rust. Single-gene resistance in western white pine (McDonald et al. 1984) and sugar pine (Kinloch and Dupper 1987) has already failed in geographically limited areas. Agricultural crop failures are

well documented in the literature, when breeders have relied on single-gene resistance mechanisms to combat native or exotic pest problems (Vanderplank 1984, 1975).

At the advent of the second generation program, we are armed with not one, but eight resistance mechanisms to achieve goals for both forest health and ecosystem management. We can for the first time simultaneously improve growth characteristics in this species. Gains achieved in improving forest health and restoring Inland Northwest ecosystems is cumulative. Gains in resistance in the first generation (Phase I and II program) are achieved via three levels of selection (plus tree, family and within-family selections). The production seed orchard selections are based on open-pollinated sources. Gains achieved in the second generation build on these existing levels of resistance through breeding, full-sib family testing and establishment of new production seed orchards.

The discussion up until now has focused on forest health goals. Tree improvement activities in all of our tree species also achieve our goals in conserving biodiversity and providing commodities in the context of ecosystem management.

White pine blister rust, shrinking land bases, competing objectives, and losses due to catastrophic fires, do not permit us to conserve populations of white pine on the "stump". Our orchards and long-term tests not only provide improved seed and information to develop tree improvement breeding strategies, but serve as gene archives or clone banks. These areas broadly sample the species' range within the context of our administrative boundaries. We also actively replenish our seed bank, which is an ex-situ approach to maintaining biodiversity. The only way we can sustain healthy, adaptable seedlings is through gene recombination (breeding), where the improved progeny can be tested against a new set of environmental conditions (e.g., changing rust hazard, climate).

De-emphasis or elimination of tree improvement in western white pine and our other four major species has it's associated risks. The ability to develop new programs also declines exponentially as our land base shrinks, as areas experience second and third generation wood removal, or as we suffer from losses due to catastrophic fires, insect and/or disease epidemics.

A brief synopsis of fine-tuning the first generation Phase II program include:

(1) Replacement of an early growth trial with a more robust and comprehensive, early selection trial.

(2) Infusion of woodsrun and Phase I seed orchard checklots, in addition to the standard crosses, to gage gains in disease resistance and height growth.

(3) Refining data collections during the first rust inspection; particularly, in the identification of no spot seedlings.

(4) Refining inoculation procedures to not only determine spore load based on number of spores delivered, but also based on germination rates of spores in an effort to achieve consistent screenings, while minimizing "escapes".

(5) Increased participation and cooperation among IETIC membership towards common goals: a) consistent supply of rust resistant white pine seed, and b) a long-term commitment to continued rust resistance development.

Major components of the second generation program:

(1) The overall breeding strategy will continue to emphasize family selection based on an index of four horizontal resistance mechanisms and within-family selection based on an individual possessing one of the four vertical resistance mechanisms.

(2) Breeding population structure consists of 340 horizontally resistant Phase II and 20 superior Phase I western white pine selections sub-divided into 18 breeding groups (sublines). Controlled-crossing occurs within, but not between sublines, to manage coancestry (related individuals).

(3) Mating occurs within the constructs of a circular, half-sib design.

(4) Assignment of a parent tree to a subline depends on a common geographic area among source material, to manage coancestry.

(5) There are four controlled-crosses per entry, 40 full-sib crosses per subline, producing a third generation breeding population of size 720 families.

(6) Rust screening is completed in a minimum of four cycles (720 full-sib families/200 families per cycle).

Second Generation Northern Region Seed Deployment Strategies (other IETIC membership may choose an alternative strategy):

(1) Seed orchard blocked by vertical resistance mechanisms. Plan to have four blocks of 7.5 acres each to meet projected Northern Region annual seedling needs.

(2) Overall orchard composition within blocks is based on 18 unique and unrelated families per vertical resistance mechanism, i.e., one full-sib family selected per subline per trait; for a genetic base of 72 families in the production population (seed orchard).

(3) Comprehensive resistance packaging favors five resistance mechanisms within one seedling, i.e., four horizontal resistance mechanisms + one of the four vertical resistance mechanisms, e.g., 4HR/Short Shoot.

(4) Deployment of 100% resistant seedlings involves equal weighting of each seedling type in bulk planting stock, e.g. 4HR/No Spot:4HR/Needle Shed:4HR/Short Shoot:4HR/Bark Reaction seedlings.

What are the consequences of not continuing the White Pine Tree Improvement Program beyond the first generation? The threat of a new virulent race of white pine blister rust would result in neutralizing our existing seed supply, since no further development of the horizontal resistance mechanisms would have occurred and the vertical resistance mechanisms would not stand up to the new virulent rust race(s). Even in the absence of no new races, we have little hope of restoring western white pine ecosystems in the Inland Northwest via natural regeneration, and certainly not in our lifetimes.

Continuing the selective breeding efforts in western white pine by the Northern Region and IETIC is the single greatest contribution we can expect to achieve with respect to improving forest health in Interior Cedar-Hemlock-White Pine ecosystems. Conserving biological diversity via the selective breeding program is also the most comprehensive approach to meeting our biodiversity objectives for this species.

A history and the current status of both the Phase I and Phase II White Pine Tree Improvement Programs (first generation) are described in the first section of this plan. A description of the second generation breeding strategy is given in subsequent sections. A summary of the test plantations and seed orchards established to date are provided in the appendices.

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THE NORTHERN REGION'S
PLAN FOR THE DEVELOPMENT OF BLISTER RUST RESISTANT
WHITE PINE FOR THE NORTHERN ROCKIES

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INTRODUCTION

Phase I Program

History. The research (Phase I) portion of the White Pine Tree Improvement Program began in 1950 (Bingham et al. 1969). From 1950 to 1975, full-sib progeny from 400 rust-free trees selected in rust decimated stands throughout northern Idaho and western Montana, were tested and measured for resistance to blister rust.

About one-quarter of the trees evaluated exhibited good general combining ability for rust resistance on the basis of higher than average survival of progeny artificially inoculated with blister rust. Resistance mechanisms were found to be present in both the foliage and bark.

Researchers were encouraged by findings that many different types of resistance were involved, with no indications of resistance inferred by a few, single dominant genes. Moreover, there was a wide and continuous range of resistance in many of the families tested. The family heritability of resistance was determined to be between 0.60 and 0.70. Resistance levels of 30 percent were observed in the F_1 generation, and it could be expected to be as high as 50 percent in the F_2s. The realized gain proved to be closer to 66 percent resistance using the 32 F_2 trees from the Moscow Arboretum Seed Orchard.

Breeding highlights. Subsequent research showed that most foliar resistance forms were accounted for by two resistance mechanisms: one causing premature needle shed and one causing the death of the rust mycelium in the shoot at the base of the needle. Each mechanism was hypothesized to be controlled by single recessive genes.

Bark-based resistance mechanisms, which result in a wide range of canker-like reactions in infected trees, were thought to be controlled by a more than a few genes.

By studying the frequency of red and yellow needle lesions it has been suggested that the classic gene-for-gene interaction is in operation in the Cronartium ricibola/Pinus monticola system. These data also provide strong evidence of the existence of pathogenic races in Cronartium ribicola.

In addition to differential types of resistance controlled by single genes, it became clear that several resistance mechanisms were at work in western white pine, i.e., lower needle lesion frequency, slow canker growth and reduced sporulation in the rust canker. These resistance mechanisms are believed to be polygenic and are not easily neutralized by new races of rust.

Nursery and field tests of F_2 material performed better than predicted, with survival significantly higher than 50 percent. In field tests in high rust hazard areas, the F_2s were found to have a much lower infection rate than the controls.

Accomplishments. A number of test plantations were established throughout the inland range of western white pine as a result of the Phase I program (Appendix G). Some of these plantations are 42 years old and suggest stability of the resistant types. The last Phase I test plantation was established in 1972.

In addition to the many test plantations, four seed orchards and a breeding arboretum were established from Phase I material. A grafted seed orchard was established in 1959 at Sandpoint, Idaho. This was the first seed orchard of any species to be established in the Northern Region. In 1989, most of the land occupied by the Sandpoint Seed Orchard was donated to the Sandpoint School District for expansion of the adjacent Sandpoint High School. Less than one acre of the original seed orchard remains under Forest Service ownership, but the trees on this land continue to be opportunistically managed for seed production.

Three additional Phase I seed orchards were established from 1971 to 1974: low-elevation (3,300 ft) orchard at the Coeur d'Alene Nursery, mid-elevation (3,300 to 4,100 ft) and high elevation (4,100+ ft) orchards at the Lone Mountain Tree Improvement Area. The relative importance of subdividing seed orchards by elevation is covered in more detail in the section on 'Improvement Strategies'. These three seed orchards along with seed collections from the combined F_1 and F_2 test at Canyon Creek, are the principal sources of seed for the Northern Region's artificial reforestation program (Appendix G).

The Moscow Arboretum was established from 1957 through 1967 with resistant seedlings and grafts of all the families tested. It was established on land leased from the University of Idaho. The USFS lease expired in 1991, and the ownership of the Arboretum has reverted back to the University of Idaho. As members of the Inland Empire Tree Improvement Cooperative, the USFS reserves the right to collect its fair share of cones from the Moscow Arboretum to meet operational reforestation needs. The Northern Region did not claim their share of cones from Moscow Arboretum for the first time in 1994, since a two-year seed need was met by our three Phase I orchards.

Phase II Program

History. The operational (Phase II) portion of the White Pine Tree Improvement Program began in 1967. The objectives of the Phase II program are two-fold: 1) to expand the testing population base by phenotypically selecting up to 3,100 new rust-resistant candidate trees, and 2) to incorporate selection and breeding for improved growth as well as blister rust resistance (Howe and Smith 1994). The Northern Region administers and provides the technical direction for the Phase II program. Technical direction is reviewed by Intermountain Research Station geneticists and the Technical Advisory Committee of the Inland Empire Tree Improvement Cooperative as needed.

From 1967 to 1969, 3,098 additional candidate trees were located by the five "white pine" Forests of the Northern Region (Clearwater, Idaho Panhandle, Lolo, Kootenai, and Flathead National Forests) other IETIC members and Commonwealth land holdings in British Columbia. Cone collections from the new candidate trees began in 1970. From 1970 to 1974, controlled pollinated cones were collected from the candidate trees. A 10-tree pollen mix from selected resistant Phase I trees was used to pollinate the Phase II candidates. Only about 500 of the 3,098 candidate trees were collected during that period. The high cost of control-pollinating wild parent trees, a complete cone crop failure in 1971, and the determination that gene frequencies for most of the resistance characteristics were relatively high, led to the decision to use open-pollinated seed for testing. In 1974, a decision was made to expedite collections by utilizing open-pollinated, rather than controlled-pollinated cones. There are no current plans to incorporate these control-pollinated

families into the existing program due to their limited genetic base (10 male parents common to all crosses).

The nursery rust screening of 3,098 candidate trees remains a challenging task. Approximately 16 cycles, comprised of approximately 200 families tested per cycle over a five year period, are needed to complete the assessment of these open-pollinated families for above average, single-gene and polygenic resistance mechanisms. Testing for blister rust resistance occurs at the Coeur d'Alene Nursery. The first cycle was inoculated in 1978. Test Cycles 2, 3, 4, 5, and 6 were inoculated in 1979, 1981, 1984, 1986, and 1988, respectively. Beginning with Cycle 7 (1990), a decision was made to initiate inoculations every year. Following this accelerated schedule, in any given year there is one cycle of test seedlings being sown, one being grown, another being inoculated, and three cycles under inspection in nursery beds.

Past experience and analysis has led to better techniques for screening and identification of above average resistant families:

- Previous inoculations (Cycles 1-6) have not produced the desired level of infection. Retesting families from these earlier cycles began in Cycle 8.

- Test size per cycle was also reduced to a maximum of 200 open-pollinated families beginning in Cycle 7 in an effort to improve the quality of the rust inspection data.

- One of the most serious problems encountered in the first three cycles of rust testing was insufficient inoculum from natural sources. As a result in 1983, a Ribes garden made up of Ribes clones proven to be good carriers of the blister rust disease, was established at the Lone Mountain Tree Improvement Area. Inoculum from the Ribes garden has been used since Cycle 4.

- Cycle 2 seedlings were predominantly primary needle. Effective inoculations and screenings must occur on secondary needles. Better growing practices were adopted at the nursery to ensure seedlings possessed secondary needles and were of approximately uniform height to ensure inoculation standards.

Overall, a fair (and in some cases inadequate) job of assessing resistance mechanisms occurred in Cycles 1-6. Inoculation and rust inspection procedures beginning with Cycle 7 have now been improved to assess the resistance mechanisms.

Early growth trials were established at the nursery beginning with Cycle 4. Previously, growth measurements were made in inoculated nursery beds. Assessment of the inadequacies of the experimental design in 1993 resulted in abandoning the Cycles 7-9 early growth trials on two accounts: 1) not enough families were planted to make selections for growth, and 2) genetic correlations between growth and the horizontal resistance mechanisms could not be estimated within these trials. Beginning with Cycle 11 (spring 1995), one early selection trial per cycle of uninoculated seedlings will be established in one of three tree improvement areas in a randomized complete block design. Final assessment of growth will be made five years from seed. Combined disease resistance and growth data of inoculated and uninoculated families (by cycle), will provide the opportunity to proceed with: (1) estimating genetic

correlations, and (2) selection at the family and within-family levels for both disease resistance and growth.

Field tests are also established for each test cycle. For Cycles 1-4 two field tests were established with uninoculated seedlings from the same families included in the nursery rust screenings, for a total of eight tests. The purpose of these tests was to observe the performance of seedlings inoculated under natural conditions in high blister rust hazard areas.

Beginning with Cycle 5 a field test called a "performance test" was established with promising looking survivors from the nursery screenings. Following completion of the nursery screening process, at least two individuals from each of the families included in the rust test were transplanted into one performance test per cycle. In Cycle 7 the criteria was generalized to include any surviving seedling with no active cankers, after the quotas for resistance seed orchard seedlings had been met. The overall purpose of the performance test is to observe the rust bed survivors under field conditions and to provide a back-up soil-based breeding orchard.

In the spring 1982, seedlings selected for production seed orchards from Cycles 1 and 2 were transplanted at Lone Mountain Tree Improvement Area (Idaho Panhandle NFs). All subsequent seed orchard selections (Cycles 3+) are established at the Grouse Creek Tree Improvement Area.

Improvement strategies. Based on the Phase I research program in western white pine and experience in breeding for disease resistance in agricultural and agronomic crops, the development of a breeding strategy for the Phase II program has been influenced by the following genetic principles (Vanderplank 1984):

- Mechanisms of disease resistance vary in type and frequency. Resistance is the observed expression of the interaction of the host genes, the disease organism genes and the environment. The level of resistance required changes with environment, weather, and disease cycle. The possibility of genetic change in the disease organism is restricted only by its own natural fitness requirements.

- Some resistance mechanisms bestow complete immunity, others slow the disease and/or curtail its development.

- Mechanisms that impart immunity are typically controlled by single genes (sometimes referred to as vertical resistance). Vertical resistance can be neutralized by a complementary gene in the disease-causing organism. Combinations of several vertical resistance genes exhibit characteristics similar to horizontal resistance.

- Mechanisms that slow or curtail a disease are typically polygenic (sometimes referred to as horizontal resistance). Horizontal resistance is not easily neutralized by new races. Resistance composed of horizontal types exhibits more stability than does resistance composed of vertical types.

These principles have led to the following breeding guidelines for developing rust resistant white pine:

- Resistance should not be based only on single-gene resistance mechanisms. While a cultivar of white pine could be developed that would be 100% immune to local races of rust, it's resistance may not last long beyond one generation in the presence of a new race of rust.

- combination of single-gene and polygenic resistance types is essential in production seed orchard seed to counter existing or new rust races.

- incorporate as many horizontal resistance mechanisms as possible into the breeding population.

Other findings that have had a strong influence on the development of the white pine breeding strategy are:

- Little geographic or elevational variation has been found in tests of populations of white pine in the Inland Northwest area (Rehfeldt and Steinhoff 1970; Townsend et al. 1972; Rehfeldt 1979; Steinhoff 1979). No zoning based on geography (latitude and longitude) or elevation is required in establishing a Phase II production seed orchard or in the breeding population (Figure 1).

- There is a fairly high juvenile/mature correlation for growth in pines. This makes early selection for improved growth possible.

- Within-family variation is present in Phase II families. A program that utilizes both family and within-family selection has the potential for significant gains.

Accomplishments and current status. New candidate tree identification and cone collections were completed in 1976. Rust screenings have proceeded up to Cycle 11 in 1995. Scion material was grafted from Cycle 7 selections in 1994 for assignment to the breeding population. This program is a joint effort of the Inland Empire Tree Improvement Cooperative, with 20 members participating. USFS seed orchard establishment and management are independent of other IETIC cooperators.

PHASE II FIRST GENERATION SELECTION STRATEGY

The selection strategy has its basis in a recurrent selection program for general combining ability. Components of this type of program involve breeding, testing and production populations (Figure 2).

Cone Collection and Seed Handling

Standard crosses. Controlled pollination continues on 14 Phase I standard cross trees with known resistance characteristics. Seedlings from these single-pair matings are included in each cycle of testing to serve as a control for: evaluating inoculation success, comparisons among Phase II test cycles, comparison among Phase I and Phase II tests, and as an aid in detecting new races of rust.

The seven standard crosses are:

17 x 293	220 x 221
19 x 70	222 x 225
20 x 21	347 x 348
208 x 314	

Open-Pollinated Seed. Details of the stand and individual tree selection criteria, marking and locating procedures for establishing the test population, are found in Appendix A. A Candidate Tree Location Record was prepared for each tree recording legals, basic measurements and cone bearing history. Copies of USFS Location Records are maintained at the Coeur d'Alene Nursery and the Moscow Foresty Sciences Lab. Other IETIC Cooperators' Location Records are maintained at the University of Idaho or by participating cooperator.

Genetic Testing

Test Cycles and Materials

Treatment. With 3,098 candidate trees to be evaluated, it is not possible to include all selections in one screening trial. Candidate trees are tested in subgroups called "cycles". Initially, cycles were larger than 200 families and comprised of families from a localized geographic area. Since some collections lagged behind other areas and this species only has one breeding unit, subsequent cycles have been made up of families from throughout the white pine breeding unit. Each cycle consists of approximately 200 families plus controls.

Controls. Seven proven control pollinations from the Phase I program have been identified as standard crosses. These crosses are included as one set of controls in each cycle of rust resistance testing. Cycle 8 inadvertently does not contain any standard crosses.

Beginning with Cycle 12 woodsrun cone collections of average performance trees are included as a checklot to determine realized gains. Minimum criterion is that trees be of cone bearing age (i.e., trees can be infected with white pine blister rust). A minimum of 30 individual-tree collections comprising the geographic range of the 200 families being tested, are bulked and sown as one lot. Each individual collection is proportionally assigned to the bulked lot based on germination tests, to ensure equal weighting. Based on the geographic location of the families per cycle, the checklot material is cycle-specific. Operational seedlots are used when available. Clearwater, Idaho Panhandle, and Lolo National Forests have recently contributed to these checklots in combination with checklots from Idaho Department of Lands and Potlatch Corporation.

Beginning with Cycle 13, a checklot of bulked seed from the Phase I Coeur d'Alene Nursery and Lone Mountain Seed Orchards will also be included in each cycle of testing to gauge our improvement in rust resistance and height growth.

Experimental Design

Seed for each cycle is pulled from the seed bank and put in stratification in January and sown in super cells in June. Historically, a target of 125 seedlings were sown per test family for the nursery screening and early growth trial. Beginning with Cycle 12 a minimum of 180 seedlings per test family and standard cross are required for the nursery screening trial (108 seedlings or

36 trees/family/rep) and companion early selection trial (72 seedlings or 24 trees/family/rep). A minimum of 15 trees/family/rep are needed in both the nursery screening trial and EST to assess horizontal resistance and growth, respectively, at the family level. A minimum of 360 woodsrun and seed orchard checklot seedlings are needed per cycle (i.e., 36 seedlings/2 plots/3 replications for the rust screening, and 24 seedlings/2 plots/3 replications for the early selection trial).

Seedlings are grown in the greenhouse the first summer after sowing. While accelerated growth occurs in the greenhouse, seedling growth is stopped in late June to mimic seedling development in a forest setting and to minimize container binding of the roots. During the first winter the seedlings remain in a breeding house maintained at 40°F. This procedure enhances the shedding of the primary needles, which are highly susceptible to blister rust and lack the desired needle resistance responses. The seedlings are grown in the breeding house during the second year to promote secondary needles and increase their size and caliper in preparation for inoculation in August.

Rust Resistance Testing

The objective of nursery screening trials is to identify families exhibiting all four horizontal resistance mechanisms and individuals within those families that exhibit one of the four vertical rust resistance mechanisms. The rust screening process is the most complex and critical procedure in the White Pine Tree Improvement Program.

Inoculation. During late August of the second growing season, when teliospore development on the alternate host is maximum, seedlings for the rust screening are inoculated. Appendix B provides a detailed account of the inoculation procedure. (Seedlings to be planted in the early selection trial are not inoculated, but remain in the breeding house until transferred to cold storage.)

Cold storage. Once the seedlings go dormant around December, both inoculated and uninoculated seedlings are tagged and packed by replication and are then placed in cold storage at 28°F until the following spring.

Transplanting. Around early May of the third growing season, inoculated seedlings are transplanted to nursery beds where they will remain for the duration of the rust screening process. Families are planted in random order within a replication. Each family block consists of seven columns of two to five rows, depending upon the number of seedlings per family, maximum of 35 seedlings per family per replication. Cycles 1-11 seedlings were planted at 6 by 6-inch spacing across the nursery bed (seven trees across) and the beginning of each family was staked and tagged with the family number. Beginning with Cycle 12 each family block will consist of six columns of six rows, with a maximum of 36 seedlings per family per replication. This strategy increases the likelihood of identifying recessive resistance traits in three more seedlings per family and makes the data collection process easier among ground crews. Seedlings are watered, fertilized and weeded as needed during their stay in the nursery beds.

Rust inspections. Inoculated seedlings are inspected for blister rust four times in the nursery beds. Seedlings are planted in May and the first inspection is made shortly thereafter, during the first week of June. The second inspection is made during September, one year after inoculation (three

months after the first inspection). The third and fourth inspections are made in September, two and three years after inoculation, respectively. A standardized set of data collected in a Cycle is presented in Table 1 using Cycle 8 as an example. Actual variables collected in earlier cycles may differ slightly.

Traits of interest. Identification of the eight rust resistance mechanisms are outlined in Table 2.

Inspection 1: evaluate needle lesion frequency (spots per meter). For each tree a five-needle fasicle is selected, needle fasicle length (mm) is measured and the number of red and yellow spots are counted. Total number of both spot colors are assessed on the entire seedling. This provides the basis to identify families with low needle lesion frequency (sometimes referred to as "spots per meter"). A second objective is to identify individuals with no spots. When a no spot seedling is identified in a plot of moderate to high needle lesion frequency, it is likely that it is a result of resistance, whereas a no spot seedling in a low needle lesion frequency plot has a higher probability of being an "escape". If 95% or greater of the inoculated seedlings exhibit needle infections it is assumed that none of the seedlings escaped infection in that plot (McDonald and Hoff 1970). The target number of no-spot trees per family plot should not exceed 5% of the total to guard against escapes and to provide a reliable estimate of the needle shed trait in Inspection 2.

Inspection 2: identify those seedlings with needle shed resistance--seedlings that had needle spots during Inspection 1, but none in the fall. Seedlings with this particular resistance mechanism will have dropped their infected needles over the summer. Cycles 9 and 10 (1993 and 1994) exhibited higher than average no spot trees, therefore, some data screening needs to occur before needle shed resistance can accurately be assessed. Seedlings are also examined for the presence of cankers. Information on canker development will help separate families exhibiting slow and fast canker growth.

Inspection 3: identify seedlings with small, early bark reaction(s) and presence of cankers.

Inspection 4: identify seedlings that had both spring and fall needle spots but never developed a canker (fungicidal shoot resistance), those that developed cankers, but canker growth has been arrested or dead or (bark reaction), those that developed a "scab" with no sign of a canker (bark reaction), and those which canker development has progressed very slowly (slow canker growth or tolerance). By the time the last inspection is made, the highly susceptible seedlings with fast canker growth will be dead.

Measurement and analysis. Data are recorded using portable data recorders (Husky Hunters) during each inspection. Intensive training is provided by the Coeur d'Alene Nursery the first day of inspection in June and again in September. Screening is done by two-person crews with frequent, periodic inspection by R1 Tree Improvement Staff to assure the accuracy of data collected. A preliminary analysis of total number of no spot trees is assessed following the first rust inspection to validate field procedures and to assess our ability to quantify needle shed resistance during the second rust inspection.

Analysis of all rust screenings per cycle is accomplished by the USFS Selective Breeding Specialist, utilizing SAS Procedures (SAS 1992) (Appendix D). Results of the rust screening process are available the year following the completion of the fourth rust inspection from Moscow Forestry Sciences Lab.

Beginning with Cycle 7, WP Analyzer Data General utility program was no longer used for data analysis because: 1) estimates of other genetic parameters were needed beyond simple family rankings, and 2) more sophisticated selection procedures (selection index) for ranking superior families and individuals were needed. When enough seed is available in the seed bank to retest Cycles 1-6 (to obtain estimates of horizontal resistance), these families will be re-evaluated using SAS Procedures criteria.

Evaluation of Growth Potential

History of early growth trials (EGTs). The EGT was targeted to provide accurate short-term growth data for ranking families in each test cycle. From an historical perspective these were the components of an EGT:

- Same families used as in the rust resistance screening.
- Completely randomized single-tree plots, 18 x 18-inch spacing, 20 individuals per family, with two border rows.
- Height (cm) measured two and five growing seasons after germination. The 5th-year measurement will coincide with the final rust inspection.
- Mean family height at 5 years was adjusted by 2-year height. Families were ranked using WP Analyzer. Damaged trees were excluded from analysis.

Not all of the cycles were planted in EGTs. As a result, only a subset of the total number of families in any given cycle was represented in an EGT. There was also no blocking in the EGTs. These design issues resulted in a limited ability to correlate growth to resistance data or to make selections based on improved growth. This approach was abandoned in favor of the randomized complete block design on farm field sites, with the full complement of families tested per cycle.

Early selection trials (ESTs). The EST replaced the EGT in 1993. Each EST will be outplanted at one of the Idaho Tree Improvement Areas: Grouse Creek or Lenore. Each EST shall be managed for optimum growth and low levels of stress. FSH 2409.26G Chapter 400 provides additional information on EST installation and maintenance and is not repeated here. These growth data will be combined with the results of the rust screening trials to facilitate correlation among the disease and growth traits for selection and orchard establishment.

Experimental design. Experimental layout is a randomized complete block design with three replications and a total of 72 individuals per family (24 individuals/family/rep). A 3 x 8 family plot is planted at 1 m spacing (3.3 x 3.3 feet).

Standard crosses will be treated like a separate family with 72 individuals per cross. Two woodsrun checklots and two seed orchard checklots will be included in each replication for a total of 360 seedlings (24 seedlings/4 plots/3 replications).

Measurement and analysis. Survival, terminal damage, baseline height, and presence or absence of rust activity due to natural inoculum are assessed in the fall of the same planting year. The final survival, terminal damage, height, and rust activity measurements will be made at five years from seed. Growth potential cannot be measured without bias in inoculated seedlings, so these data will be used to provide estimates of heritability, gain and family rankings for growth. Type B genetic correlations among growth and disease resistance will be determined between the EST and rust screening data. Once these EST data are analyzed, the EST will serve as a source of scion material for breeding orchard establishment. When the need for vegetative material has been satisfied, the test will be eliminated. Using scion from an EST for production seed orchards is not recommended since these trees have unidentified vertical resistance mechanisms, with an overall resistance level of only 30%.

Long-Term Field Tests

Seedlings for the field tests come from excess material in the rust tests that are canker-free at the time of selection. Field tests are established the spring following the fourth rust inspection. These tests are established on typical white pine sites to provide a measure of family and individual performance over a period of about one-half rotation length. The field tests also serve as clone banks and if the need arises, as a soil-based breeding orchard. Characteristics measured are survival, terminal damage, growth, and rust resistance.

Experimental design. The trees from Cycles 1-7 were planted in a completely randomized layout with non-contiguous, single-tree plots at a spacing of 10 x 10 feet. Beginning with Cycle 8, a randomized complete block design will be implemented to account for some of the environmental variation. The predominant blocking factor is aspect. Seedlings from any given family will be divided as equally as possible among three blocks. All tests are surrounded by two border rows. From a design standpoint the test will be unbalanced at the onset (i.e., unequal number of trees per family per block), but those data can still be analyzed using plots means and/or general linear models.

Measurement and analysis. Measurements will be made at age 5, 7 and 10, and at 5 year intervals, thereafter. Traits of interest are: survival, terminal damage, presence/absence of branch and bole cankers, number of stem cankers (only in high survival tests), total height (dm), and possibly diameter (mm).

Families will be ranked by least square estimates of mean height, number of cankers per tree, percent clean, and survival (based on plot means), using SAS procedures. Results of these analyses will also be used to prioritize retesting of Cycles 1-6 families for horizontal resistance.

Seed Orchard Establishment

While one seed orchard could supply all of the seedlings needed throughout the Inland range of western white pine, more than one seed orchard will be established as protection against the possible loss of an orchard, and to aid

in supplying a continual seed source of genetically improved seed when transitioning between two generations of tree improvement.

The White Pine Tree Improvement Program is a cooperative effort, where data and vegetative material are made available to interested cooperators for their seed orchard establishment. Scion and cuttings may be collected from superior donor trees once the trees have survived transplanting shock and exhibit good growth and vigor, which is typically three years after transplanting. No scion or cuttings are made prior to transplanting the 3-2 seedlings from the nursery beds to the Grouse Creek Tree Improvement Area. Excess seedlings in early selection trials (those seedlings not targeted for the breeding orchard) are available to white pine cooperators for their use immediately following the analyses, i.e., no three-year time delay.

Selection Strategy

Cycles 1-6. At the completion of the fourth rust inspection in nursery beds and the final height measurement in the early growth trials, families were ranked for low needle lesion frequency, slow canker development, and height growth. Families scoring in the lower 30 percent for height growth were eliminated from further consideration, regardless of their performance in the rust resistance test. For those families that ranked high in all three categories, individual trees were selected in rank order that exhibited the following differential resistance characteristics: 1) no spots, 2) needle shed, 3) fungicidal shoot, and 4) bark reaction.

An equal number of seedlings were selected in each resistance category. The individual selected must have had average or better height growth when compared with other individuals selected. No more than two individuals of a particular resistance type were selected from any one family for any one seed orchard. Occasional "wildcard" trees were selected from low scoring families if the selected individual was truly outstanding in one or more of the selection criteria. Orchard selections are planted at the Grouse Creek Tree Improvement Area, Idaho Panhandle National Forests.

Cycles 7-16. At the completion of the fourth rust screening, families are selected for outstanding performance based on the four horizontal resistance mechanisms (Table 2) using a selection index. Weightings for the 'a' vector are (4, 3, 2, 1) for bark resistance, needle lesion frequency, early stem symptom appearance, and tolerance, respectively. Index scores (I) are determined for each family based on the following equation:

$$I_F = b_1P_1 + b_2P_2 + b_3P_3 + b_4P_4$$

where, the four regression coefficients (b_1-b_4) are solved to maximize gains among the horizontal resistance mechanisms. This method is comparable to that of Hoff et al. [in prep].

The primary difference between these two selection indices occurs at family rankings greater than a rank level of 15, where the selection index coefficients applied to those data identify superior families with good bark reactions, but slightly higher needle lesion frequency (spots per meter) than Hoff et al. [in prep]. The top 20 and top 30 out of 200 families are selected per cycle for the production seed orchard population and breeding population, respectively. Each cycle of data will be analyzed by both index methods to confirm the stability of family rankings.

The within-family selection strategy among the top 20 seed orchard families is selection of equal numbers of vertical resistance mechanisms per cycle, i.e., 50 no spot, 50 needle shed, 50 fungicidal short shoot, and 50 bark reaction trees. Since only 10 trees are selected per family, it is inherently impossible to select equal numbers of seedlings per family by resistance mechanism (10/4=2.5 trees). In addition, when the the total number of seedlings of a particular vertical resistance mechanism exceeds a count of 50, seedling heights are then used to rank the top 50 seedlings. Seed orchard layout concerns are discussed below.

Seed Orchard Design

First generation seed orchards are established by an advancing front concept as selected materials become available at the completion of each cycle of testing. There are three major design issues: orchard design among four vertical resistance mechanisms, minimizing inbreeding by maximizing distances between related individuals, and cone collection/processing deployment strategies for tree planting.

There are two primary alternatives for seed orchard design:

(1) Randomly mix the top 200 individuals from each of the 20 families per cycle (resistance level = 60%), or

(2) Systematically block the seed orchard by the four vertical resistance mechanisms (resistance level = 100%) (Seed Production column, Figure 2).

Other strategies not considered in this breeding plan favor development of just horizontal or just vertical resistance mechanisms, with resistance levels at about 30% and 60%, respectively.

Evaluation of alternatives. Alternative 2 provides the maximum gain and the potential for 100% white pine blister rust resistance. Seed coming out of each block would have the added insurance of above average horizontal resistance (30% range) to guard against potential changes in the virulence of new rust races, which would neutralize the single gene resistance but not the polygenic resistance mechanisms.

Choice of alternative 1 or 2 is dependent upon the currently accepted models of gene action among the four vertical resistance traits. The hypothesized gene models are being verified in two independent studies being performed by the White Pine Tree Improvement Group of IETIC and USDA-Forest Service, Northern Region. The IETIC study is evaluating needle shed, short shoot, and bark reaction families utilizing controlled-crosses of pollen mixes for each resistance mechanism.

The USFS Vertical Resistance Study involves full-sib crosses (20 crosses per trait), as well as the additional control of selfing to eliminate any noise (e.g., pollen contamination) in the expected proportions from the full-sib crosses. There are 20 planned selfs per trait. There are no planned crosses between any two resistance mechanisms, e.g., bark reaction x needle shed. There are two few no spot trees among the Phase I F_2 material to test the hypothesis of single gene resistance.

A disadvantage of alternative 2 is an extended testing program to identify enough individuals to make up each orchard block versus taking the first available superior seedlings and clonally replicating them to construct an orchard. Another potential disadvantage is the ability to construct the no spot seed orchard block. The identification of no spot formation in secondary needles (no spot trees) is highly dependent upon the inoculation procedures. As a result, we are presently limited in our ability to identify large numbers of no spot seedlings, i.e., identified five seedlings in Cycle 7 and 28 in Cycle 8. (The target number of no spot seedlings per test cycle is 50.) Inoculation procedures are continually being fine-tuned and precautions are being taken during measurement and analysis to reduce the frequency of classifying an escape as a no spot seedling. A shortage of no spot seedlings is not anticipated to be a long-term problem.

The major advantages of alternative 2 are the potential for 100% resistant material and a large genetic base (20 families x 4 orchards) in the production seed orchards.

If a cooperator does not wish to wait for the results of the vertical resistance studies and/or does not agree with the hypotheses of single-gene recessive inheritance, alternative 1 is a valid option. A possible scenario is a clonally replicated orchard with the first available families and reduced costs of managing one orchard versus four orchard blocks. The major drawback however, is if seed orchards selections are randomly assigned to a seed orchard layout, resistance levels will not exceed those reported for the Moscow Arboretum Seed Orchard (60%).

Reduction in resistance levels occurs as a result of: 1) individuals being randomly assigned by resistance level to an orchard (alternative 1), or 2) pollen contamination from non-orchard trees (alternatives 1 and 2) or pollen contamination from an adjacent resistance block (alternative 2). Consider the following example to illustrate reduction in resistance from 100 to 60%:

Vertical Resistance Mechanism	Resistant Genotype	Susceptible Genotypes
No Spot	nn	NN, Nn
Needle Shed	ss	SS, Ss
Short Shoot	ff	FF, Ff
Bark Reaction	bb	BB, bb

Genotype of resistant orchard donor: nnSSFFBb (100 % resistant)
Genotype of second resistant orchard donor: NnSsffBB (100 % resistant)

Focus on just two of the traits, no spot and short shoot, and their progeny produced from a random design or due to pollen contamination in a blocked orchard displayed in a Punnett square:

Parental Genotype	Gametes Produced	Progeny Produced		
			nF	
nnFF	all nF	Nf	NnFf	Only the nnFf progeny
Nnff	Nf and nf	nf	nnFf	are resistant, yet both
				parents were resistant.

For completeness, all four traits within an individual would need to be considered simultaneously, to determine the overall reduction in resistance by randomly assigning resistant genotypes within an orchard. It is left as an academic exercise to determine the overall resistance of the 3^4=81 unique parental genotypes, assuming equal genotypic frequencies.

What is the maximum risk between alternative 1 and 2? Even if the hypothesized vertical resistance mechanisms are not confirmed as single-gene mechanisms, the blocked orchard can be managed as a wind-pollinated orchard with a large genetic base and resistance levels around 60%. However, if the orchard is not blocked from the beginning (alternative 1) and the single-gene hypotheses is confirmed, then the only way to achieve 100% resistance would be: 1) through controlled crossing each seed orchard entry by resistance mechanism, 2) by developing a new (blocked) orchard, or 3) vegetative propagation of resistant individuals.

Forest Service. The Forest Service has adopted alterative 2 and began implementing this strategy with Cycle 7 seedlings. Other members of the IETIC can partly overcome some of the limitations of designing and implementing a seedling seed orchard, by establishing clonal seed orchards from the seedling donors.

It is important to note that orchard blocks established from forward selection (selecting progeny from the plus trees in the rust beds) is the only way to achieve gains in vertical resistance (along with horizontal resistance). Orchards established based on backward selection can only contribute to gains in horizontal resistance since the plus trees themselves never went through the rust screening process. Gains in vertical resistance are theoretically possible with backward selection, but are unknown without further testing of the parent trees.

Approximately eight more cycles of rust resistance testing are needed to complete the Phase II plus trees, including retesting of promising families from Cycles 1-6. At that time there should be approximately 2,000 trees in the Grouse Creek TIA, which have been selected for both horizontal and vertical resistance. There will be about two acres of Forest Service seed orchard established from each cycle of testing (10 individuals from the top 20 families). The trees are planted at a 20 x 20-ft spacing, which is about 109 trees per acre (TPA). Approximately 30 acres of white pine seed orchard are needed to meet projected annual seedling needs for the Forest Service. To meet this goal, grafting of selected material will have to occur to make up the balance of the acres needed: 2,000 seedlings at 109 TPA = 18.5 acres + 1,254 grafts at 109 TPA = 11.5 acres) for a total of 30 acres.

With either alternative 1 or 2, there will be related individuals in a seedling seed orchard, since 10 trees are selected per family per cycle. Efforts to maximize the distance between relatives within an orchard block will be achieved by using SOMAD, Seed Orchard Mapping and Design Software (Marsh 1982, 1985) at Grouse Creek Tree Improvement Area and Coeur d'Alene Nursery.

Improved seedlings will be packaged for field outplanting by mixing equal numbers of seedling produced by each orchard resistance block. Theoretically, a planting site would be comprised of no spots, needle shed, short shoot, and bark reaction seedlings.

A backup seed orchard at CDA Nursery (for the Grouse Creek Seed Orchard) is discussed below in the section titled "1.5 Generation Orchard Option".

IETIC Cooperator orchards (non-Forest Service). Orchard composition in this case is based on a clonal seed orchard, where less than 200 families can be selected with more ramets per clone (family), to meet a Cooperator's annual seedling needs. This is demonstrated in the following example of 100% resistance seed orchard blocks under alternative 2:

a). Forest Service has 200 unique families among 500 seedlings per orchard block (i.e., 20 families x 10 seedlings x 10 cycles (7-16) / 4 blocks).

b). An IETIC Cooperative orchard could have 25 unique families per block, 20 ramets per family, for 500 seedlings in each orchard block.

If alternative 2 is chosen, it is recommended that a minimum of 20-30 different families be selected per vertical resistance mechanism, to ensure a broad genetic base for operational plantings. Since time is part of gain, there is the option to immediately choose the first 20-30 families as they become available at the completion of a cycle, wait to choose the top 20-30 families overall after Cycle 16 has been completed in 2003, or some combination thereof.

1.5 Generation Orchard Option: An orchard is referred to as a 1.5 generation orchard when the best individuals from a first generation program are used to construct a new orchard without first proceeding with controlled pollinations to generate a new testing population. In order to achieve the best resistance levles, both the Phase I orchards at Coeur d'Alene Nursery and Lone Mountain TIA will need to be phased out as soon as white pine seedling needs are met at Grouse Creek Tree Improvement Area. Replacement of the Phase I seed orchards can occur one of two ways:

1) Phase II first generation, "1.5 generation" clonal orchard, or

2) Second generation combined seedling and clonal seed orchard.

Option 1 has several advantages. The blocked seed orchards at Grouse Creek Tree Improvement Area need to be replicated at another location as insurance that Phase II elite genotypes are not lost due to fire, wind, vandalism, etc., and to ensure a sustained seed supply. Problems encountered in an advancing front orchard (like Grouse Creek) can be overcome in a 1.5 generation orchard since a new blocked orchard can be designed with the best material (rust screenings confirmed by long-term tests) at the end of Cycle 16. Also, if Forest Service projected seed needs changed after the year 2000, the 1.5 generation orchard acres could be adjusted to reflect the new seed needs. Lastly, if for any reason the hypothesis of a single-gene recessive model is not verified in the vertical resistance studies, an opportunity to redesign a random (no blocking) seed orchard could receive further consideration.

Option 1 is preferred by the Forest Service since the top priority is to ensure that the Phase II elite material be replicated at another location. It is anticipated that the existing Phase I seed orchard at the Coeur d'Alene Nursery will not be replaced with a 1.5 generation orchard until Cycle 16 rust screenings have been completed and the Northern Region's seed needs are being met at the Phase II blocked orchards at Grouse Creek Tree Improvement Area.

Lifting and Transplanting

Selected seed orchard individuals are tagged with pedigree, bed, row, and column designations in the nursery bed, and type of vertical resistance (since inoculations occur with two-year old seedlings, it is not possible to identify more than one vertical resistance mechanism in any given seedling). The 3-2 trees are lifted bareroot with a tractor-powered lifter. Roots are pruned to 12-14 inches in length, sorted by resistance mechanism, placed in bags, and stored in the coolers at the nursery to await transport to the seed orchard site. The outside of the bags are labeled with bag number and resistance mechanism (as in 1 of X, Needle Shed).

Planting holes are dug with a 12-inch tractor-mounted auger just prior to planting. Shaping the hole and filling in around the planted trees is done with a shovel. Trees are watered immediately after planting. Subsequent fertilization and watering are outlined in Appendix F and in the Grouse Creek Seed Orchard Management Plan.

Maintenance

A seed orchard management plan has been prepared for each white pine seed orchard. This plan incorporates management activities for white pine seed orchards described by Hoff and Coffen (1982) and is under revision to incorporate recent findings that promote early and abundant flowering. Lastly, cones will be collected and processed by resistance mechanism, no bulking will occur in the seed orchard(s). Operational seedlings (usually 3-0 stock) will be sown and grown by resistance mechanism, and bulking will occur during the lift and pack phase so that operational plantings or restoration projects will consist of equal mixtures of 100% resistant material. This strategy is preferable to bulked lots of unequal weightings, since it makes it more difficult for a new race of the pathogen to get a foothold, because it will have insufficient fitness to neutralize the existing resistance mechanisms (Vanderplank 1984).

SECOND GENERATION BREEDING

Introduction

Development of a second generation breeding strategy focuses on several issues: breeding population size and structure, mating design, test design, and seed deployment strategies. This section of the plan was originally highlighted as the "advanced generation breeding strategy". Realistically, it is undesirable to make firm plans any farther in the future than one generation of improvement (Kang 1979b, White et al. 1993).

Theoretical studies show that breeding populations with effective size of 20 to 50 members may be large enough to sustain a selective breeding program for several generations (Robertson 1960, Baker and Curnow 1969, Kang 1979a, Nicholas 1980, Mahalovich and Bridgwater 1989, Bulmer 1985, White 1992). Justification for larger breeding populations are warranted if: 1) effective population size is less than census number, 2) the breeding population(s) serves a dual function of meeting both breeding and genetic conservation objectives, 3) many traits are being bred a program, and/or 4) intensive

efforts to achieve large gains from (family) selection are sought in the first few generations.

In advanced generation programs a large mainline breeding population is typically subdivided into one or more structures. There are three main types of breeding population structures. First, are small multiple populations made up of 20-50 individuals, where different selection objectives are employed within each subpopulation. Selective breeding objectives can emphasize different traits among populations and/or developing site-specific improved families when there is evidence of large genotype-by-environment interactions (Namkoong 1992, Burdon and Namkoong 1983).

The second type of structure within the context of an heirarchical breeding program, typically has one large breeding population (100+ individuals) and one or more nucleus populations concentrating the very best breeding orchard selections into these elite groups of size 10-50 (Mahalovich 1990, Cotterill et al. 1988). Short-term gains can be advanced quicker than can be done within one large breeding population. The large mainline population serves the dual role of meeting genetic conservation objectives and sustaining long-term gains. Positive assortative mating has often been employed within these nucleus or elite populations to further increase gains (Mahalovich 1990, Mahalovich and Bridgwater 1989).

Lastly, sublines may be adopted to facilitate management of inbreeding. At a first glance, sublining may look like small multiple populations; however, sublines are replicate populations, i.e., the same objectives are common among sublines (Burdon and Namkoong 1983). Selections within a subline are unrelated to any other subline to allow complete avoidance of inbreeding in a wind-pollinated seed orchard. This is achieved by establishing only one selected family from each subline in the seed orchard.

All three types of breeding population structures have increased in popularity in advanced generation programs, over the classical approach of using one large breeding population. The ability to carry a large population (over 200 unique selections) can be administratively prohibitive. Smaller total breeding population sizes coupled with horizontal structure (breeding groups or sublines) meet the objectives of managing inbreeding, while providing flexible options to increase short-term gains. Moreover, forest trees have not been through several generations or centuries of domestication as is the case in major agricultural and agronomic crops, nor has line breeding been practiced, both of which can result in the infamous accounts of increased mortality, reduced vigor, loss of fecundity, and sterility.

The scope of the White Pine Tree Improvement Program has a large enough breeding population to meet the needs of long-term breeding and genetic conservation objectives, utilizing many traits, and a desire to achieve gains from family selection (horizontal disease resistance) in the first few generations. This plan outlines developing a breeding strategy based on four horizontal resistance mechanisms, four vertical resistance mechanisms, and possibly growth. Improvements in growth however, will not be at the expense of any loss in disease resistance or increased susceptibility. In the crop and agronomy literature, breeding strategies are generally based on only one qualitative or one quantitative trait, particularly if disease resistance is involved (Vanderplank 1984). With western white pine we face a challenge to develop a generalized breeding strategy utilizing up to nine traits, which are probably a mixture of both qualitative and quantitative mechanisms. Moreover,

the breeding strategy has to be flexible enough to allow any one IETIC cooperator to develop their own selective breeding program, independent of the mainline Northern Region program, which has been developed to meet the overall needs of all IETIC Cooperators.

As with the first generation tree improvement program, the most conservative approach is to assume the production population or seed orchards of cooperators will likely remain wind-pollinated. This assumption also guides the choice of advanced generation breeding population structures.

The overall western white pine second generation breeding strategy will continue to emphasize family selection based on an index of four horizontal resistance mechanisms and within-family selection based on an individual possessing one of the four vertical resistance mechanisms. When the number of vertical resistance mechanisms exceeds the desired per cycle target, individuals will then be ranked based on height growth.

Agreement Among Cooperators

In support of the revised Master Memorandum of Understanding (1977), there will continue to be a free exchange of genetic material and genetic data among IETIC members. Breeding, selection and testing will be a cooperative effort among all membership. Production populations (seed orchards) for operational planting programs will be developed individually or jointly for non-USFS cooperators. The USFS Northern Region seed orchard program will remain independent of other IETIC members or the White Pine Species Group. USFS will take the lead on clone bank establishment of the breeding population to meet in part, the objective of genetic conservation. Phase II field tests managed by individual cooperators will all serve as genetic archives.

Breeding Population Size and Structure

The core of this tree improvement program focuses on 3,098 plus tree collections in the first generation, testing their open-pollinated progeny, followed by selection of the top 340 families for inclusion into a breeding program. The balance of the breeding population is comprised of the best 20 Phase I full-sib families, to further capture those Phase I gains in the second generation program.

At this writing, we are just beginning to collect growth data in the rust beds beginning with Cycle 8, and in independent early selection trials beginning with Cycle 11 material. Genetic correlations among the disease resistance traits and/or growth are pivotal to making a decision among the array of breeding strategy alternatives. For multiple population breeding it is especially advantageous to have two traits of interest genetically uncorrelated, as is the case in Pinus elliotti, between growth and fusiform rust resistance (White et al. 1993).

Hoff and Steinhoff (1980) reported no siginificant (phenotypic) correlations between resistance types and growth in 12, 13 and 14-year old inoculated white pine trees planted in a field test. The first genetic correlations among the four horizontal resistance traits with growth were derived from Cycle 8 nursery data (Table 3b). These estimates are only preliminary indications of genetic correlations since they were made on inoculated seedlings and as a result, growth potential may be limited by rust infection. The overall sample size for

this analysis was substantially reduced due to rust mortality by the fourth inspection (Mahalovich in prep.). The four genetic correlations are favorable, however, in that there is an increase in height for reduced early stem symptoms and needle lesion frequency (r_G = -0.24 and -0.61, respectively) and increased height with more bark reactions and increased tolerance to the rust (r_G = +0.18 and +0.45, respectively). Subsequent genetic correlations derived from the nursery data and early selection trials will be used to finalize the choice among the four proposed breeding strategies.

About one-half of the first generation testing has been completed. Grafting of the breeding population began in 1994 in preparation for the second generation program.

Often in advanced-generation tree improvement programs, the breeding population will not be managed as a single, unified population. Rather, the population will be horizontally and vertically subdivided for one or more reasons: 1) to facilitate placing more emphasis on members with a higher genetic quality, 2) to allow different segments of the population to be bred for different selection objectives (e.g., traits, environments), and 3) to manage inbreeding in the production population by subdividing the breeding population into sublines (White et al. 1993).

The multiple population breeding approach in the 1992 program review (Namkoong 1992) was not given further consideration in western white pine for the following reasons. No genotype-by-environment interaction in white pine has been reported in the literature, which would have suggested a need to match genotypes to site. For blister rust resistance there is no apparent advantage to developing different lines for low or high hazard sites: the goal is to develop resistance levels that achieve the desired results on all sites regardless of hazard rating. Production seed orchards are still targeted to be managed as wind-pollinated orchards; however, pollen management will be stressed in USFS orchards to realize expected gains in disease resistance. In addition, there is some indication that the superior open-pollinated families may be related, e.g., the top 20 seed orchard families from Cycle 7 are common to three geographic areas, with 11 of the 20 selections within two sections of each other (Mahalovich in prep). Therefore, a strategy for western white pine that manages any suspected relatedness (to ensure outcrossed progeny from wind-pollinated seed orchards) favors a sublining approach over one large main-line population or multiple population breeding.

Breeding Strategy Alternatives

Issues of concern in evaluating design of the sublines include:

- achieving expected gains based on the hypotheses of advancing both single gene and multiple gene resistance traits,
- management of inbreeding in relation to the deployment of selected material to a second generation seed orchard (discussed below),
- conserving genetic diversity within the total breeding population beyond the second generation program, and
- assigning parents to sublines at random to increase within-subline genetic variance to achieve greater expected genetic gains (Bridgwater et al. 1993).

The goal is to produce 100% resistant seed from blocked orchards incorporating both horizontal and vertical resistance mechanisms. The use of sublines for western white pine are evaluated in the context of three alternatives:

1. Breeding alternative B1: advance gains among 4 horizontal resistance traits. The top 340 Phase II families are selected by an index of four horizontal resistance mechanisms, and these families are also assigned at random to sublines based on a common geographic origin. All five traits are advanced within each of 17 sublines. The top 20 Phase I families are assigned to the 18th subline. Any given family would be represented in only one subline.

2. Breeding alternative B2: advance gains among 4 horizontal resistance traits and growth. The top 340 Phase II families are selected by an index of four horizontal resistance mechanisms and possibly growth. Within-family selection is based on individuals expressing superior growth performance. The top 20 Phase I families are included based on their rust screening and field test performance.

3. Breeding alternative B3: advance gains among 4 horizontal and 4 vertical resistance traits and growth. The top 340 Phase II families are selected by an index of four horizontal resistance mechanisms and within-family selection is based on both vertical resistance mechanisms and growth.

Evaluation of Breeding Strategies

Time: Breeding strategy B3 appears to be the most comprehensive, allowing for gains in up to nine traits based on their levels of heritability, additive variance, and genetic correlations. B3 however, would result in a minimum of a 15-year time delay to wait for the within-family selections to reach reproductive maturity for breeding. Crosses in the breeding population would not begin before the year 2008, and it is therefore undesirable alternative (time is part of gain). A variation of alternative B3 is being considered as the advanced generation breeding plan for sugar pine in Region 6 (Sniezko, in prep). Alternatives B1 and B2 can be implemented as soon as families have completed their five-year resistance (and growth) testing phase. The timeline incluces relocation of the original plus trees, collection of scion and pollen, grafting, followed by controlled-pollinations the following year.

Optimize gains and minimize risk: The major concern is to minimize the risk of developing a breeding strategy when there is incomplete information on the genetic mechanisms for each disease resistance trait and their relationship to growth, both of which are presently being investigated. Given that the superior plus trees have yielded sufficient numbers of vertical resistant seedlings in the rust screenings, it is suficient to collect scion from the parental trees based on their horizontal resistance levels (without having to rely on vertical resistance as well). Alternative B3 is not considered further.

The primary choice is between alternatives B1 and B2. Since the primary goal is achieving disease resistance, alternative B1 is chosen for the second generation program. Alternative B2 may be considered in the future when more information becomes available from the white pine studies presently under investigation.

Under the selected alternative B1, the main-line breeding population will be horizontally subdivided to have a minimum target of 15 sublines (replicate populations), with approximately 15-20 individuals per subline. Two additional sublines will be developed from superior Phase II field test selections from Cycles 1-6, but this addition will require further evaluation to meet minimum resistance criteria discussed in the next section. The last subline will be comprised of 15-20 Phase I selections, for a total breeding population size of 360.

Tree Selection and Identification

The selection criteria for production seed orchard establishment includes both horizontal and vertical resistance mechanisms. The selection criteria for the breeding orchard population includes horizontal resistance as a minimum and opportunistically capitalizes on vertical resistance when data are available. Scion and pollen collections for subline development under alternative B1 are dervived from the following sources:

1) Backward selection: Phase II first generation plus trees identified as elite families in the rust screening.

2) Backward selection: Phase I first generation elite trees from Moscow Arboretum, Hog Meadows (Phase I F_2) or Canyon Creek (Phase I F_1 and F_2) field tests (once the presence and level of horizontal resistance are identified among high ranking trees).

3) Forward selection: Phase II first generation seed orchard donors and Cycles 7-16 field test trees.

4) Forward selection: Phase II first generation Cycles 1-6 field test trees (once the presence and level of horizontal resistance are identified among high ranking trees).

The largest component of the breeding population (sublines 1-15) are from selected families identified in the Phase II rust screening trials. The proportion selected is 0.15 or the top 30 families out of 200 test families per cycle. Scion and pollen are collected from the surviving top 30 parent trees in any given cycle. There is a planned total of 300 backward selections from Cycles 7-16 (seed from Cycles 1-6 is being retested in Cycles 7-16). In the event of a dead or missing parent tree, every effort will be made to represent that family with material in the field tests (forward selection).

Phase I families and promising Phase II families from Cycles 1-6 field tests will be used to construct up to three more sublines: one subline from Phase I families and two sublines from Phase II Cycles 1-6 families. Because of the narrow genetic base from the Phase I testing program, no more than one subline can be constructed to minimize relatedness among sublines.

Prior to the development of these three sublines, there needs to be some confidence in the levels of horizontal resistance among the selections. This can be achieved by two methods. First, retesting the seed from selected families in the rust screening trials will provide the best estimates of the horizontal resistance mechanisms (as is the case with families tested in Cycles 1-6). In the event that a family no longer has enough seed in the bank for retesting, the second approach will evaluate a family in an existing field

test. Progeny test performance (presence/absence of rust, percent clean, and number of cankers per tree) will be processed through a rust index equation for individual hosts (McDonald et al. 1995) to determine a breeding value. It is proposed that the IETIC White Pine Tree Improvement Group take the lead on identifying superior families in field tests using the rust index, to accelerate construction of these two sublines from Phase II field tests.

The possible challenges of retesting Phase I and Cycles 1-6 Phase II families are insufficient quantities of seed in the bank for retesting and/or the original plus tree may not be available in the field for recollection. The primary advantage however, of using this material for three more sublines is that scion and pollen may be readily collected from the Phase I and II field tests. It is easier to collect scion and pollen from these field tests than from the original parent trees in excess of 80 feet. Moreover, it may be possible to capture some vertical resistance in these breeding orchard donors. In this case, additional gains could be achieved in constructing the breeding population via forward selection.

Of special note, under no circumstances will the previously identified at-large or wildcard trees be used for inclusion in the breeding population or subsequent seed orchards in the second generation. At-large trees are those putative "outstanding" individuals in field tests that were not established as an inoculated seedling from a rust test bed. No information is available on the horizontal or vertical resistance mechanisms in at-large trees. Wild card trees are putative "outstanding" individuals from families that performed poorly in rust and/or growth tests.

Utilizing at-large trees is comparable to mass selection--phenotypically selecting plus trees from the field and assigning them to a breeding population with no testing to estimate their general combining ability (presence of disease resistance genes and good growth genes to pass onto the next generation). Using wildcard trees contaminates gains since the individual comes from a poor performing familiy. In addition, an outstanding individual within a poor performing family is probably an example of a tree growing under good environmental conditions (low rust hazard, no limiting factors), and/or being a good specific combiner. Good specific combiners are due to the unique combination and arrangement of genes from two (sometimes poor performing) parents. A good specific combiner in a breeding program can only pass on its genes, it cannot pass on its good combinations and arrangements of genes.

In summary, the second generation breeding population will potentially be comprised of both Phase I and II parental genotypes (backward selection) and their progeny (forward selection).

Clone Bank and Gene Archives

There is a three-fold strategy for conserving genetic diversity in the White Pine Tree Improvement Program. Following successful completion of the controlled crosses within a subline, the two healthiest grafts from each entry will be transplanted to a USFS tree improvement area in north Idaho. Excess breeding parent material will be offered to interested cooperators and/or recycled to make room in the breeding house for other breeding activities. Establishment and maintenance of the western white pine clone bank will be the responsibility of the Forest Service.

Phase I and II field tests should continue to be managed for long-term test objectives, and additionally as gene archives or genetic libraries "on the stump". Test maintenance among IETIC membership, including periodic remonumentation to track identification over time, would remain the responsibility of the plantation manager and associated land owner.

The final component of the genetic conservation strategy is implemented when transitioning between establishment of the first and second generation seed orchards. The two best seedlings in each family for each vertical resistance mechanism from the first generation seed orchards are to be simultaneously grafted into the clone bank. This strategy involves the top 200 Phase II families at Grouse Creek Tree Improvement Area, for a total of 400 grafts. The timing of establishing this section of the clone bank isn't critical, but all grafting should be completed before the second generation seed orchards replace the Phase I and Phase II first generation seed orchards. The total clone bank will be made up of two grafts per 360 subline entries and 400 grafts from the production seed orchards, for a maximum of 1,120 grafts.

Genetic Testing

Introduction. The design of genetic tests in advanced generation breeding is dependent on two primary components: the mating design and the information required to sustain a recurrent selection program for general combining ability. The test design serves the purpose of 1) ranking families, 2) selecting families and/or individuals, estimating genetic parameters, and 4) demonstrating genetic gain (Zobel and Talbert 1984). Any test design will not be optimal for all purposes (Huber et al. 1992).

A complementary mating and testing design is not considered a worthwhile approach in western white pine, because the method used to screen for disease resistance is not comparable to estimating breeding values for growth in other conifers (White et al. 1993, McKeand and Bridgwater 1992). This advanced generation mating and testing strategy in slash and loblolly pines entails polymix progeny tests for estimating family breeding values in replicated tests, along with full-sib crosses developed for within-family selections in unreplicated outplantings. Because of the nature of screening for disease resistance in white pine, family and within-family selections can only be identified in the rust screenings.

Unless another screening process is developed for identifying rust resistance mechanisms in seedlings or in mature plant tissue via molecular genetics, second generation testing will continue to hinge upon the artificial inoculation and nursery rust screening procedures employed in the first generation program. An unbalanced test design for nursery rust screenings was not given further consideration since the randomized complete block design adequately serves to address the four genetic test objectives in an operational tree improvement program. (Sub-sampling 15 individuals per plot for estimating horizontal resistance mechanisms can be considered an unbalanced test design.)

Experimental design. The randomized complete block design will continue to be utilized for second generation field testing. Five checklots will be used in both the nursery trials and field tests. Each of these bulked checklots will occur in two plots or entries in each replication. The composition of each checklot is discussed below:

* Phase I bulked lot from Moscow Arboretum.

* Phase I bulked lot from both the Coeur d'Alene Nursery and Lone Mountain Tree Improvement Area white pine seed orchards.

* Phase II bulked lot among the four resistance mechanisms, from Grouse Creek Tree Improvement Area.

* Woodsrun bulked lot covering the geographic range of the entries among the sublines being tested in any given cycle. As a result, this woodsrun checklot is expected to differ among second generation cycles.

* Second generation checklot to compare the performance both among sublines and between cycles. This checklot will be developed through polycross procedures.

The testing program will also include the Phase I standard crosses as a control to monitor changes in the rust population over time. Each of the eight standard crosses will be treated as a full-sib test family, i.e., one plot or entry per replication.

The testing and inoculation procedures for the second generation program are identical to the Phase II first generation program, with the exception of testing full-sib families instead of open-pollinated families. Possible design improvements when costs and space are not limiting factors include replicating rust screenings in time, increasing the number of replications from three to four blocks, and testing more individuals per family to be able to identify more vertical resistant progeny. To reduce the costs of test establishment and maintenance, crosses from more than one subline can occur within a nursery screening trial or field test location. Test establishment and ownership will be based on interest levels among IETIC membership.

Mating Design

Choice of mating design depends on the need to: provide a genetic base for the next generation of selection, estimate genetic parameters, preserve effective breeding population size, and meet seed orchard roguing concerns. The first three objectives are of primary concern in western white pine.

Kang and Namkoong (1979, 1980) examined expected rates of allele losses and concluded a balanced mating design (i.e., equal representation of all parents) is more important than mating design type, in minimizing rates of allele losses. Mating design alternatives were evaluated based on efficiency, Type B correlation genetic ratios, and precision (Huber et al. 1992). Efficiency (i) is a function of the individual observations in an experiment lowering the variance of parameter estimates e.g., single-tree heritability. Type B genetic correlations are derived from two traits measured on different individuals (Burdon 1977) e.g., disease resistance in a nursery rust screening trial and growth in an early selection trial. Type A genetic correlations are more common, where both traits of interest are measured on the same individual, as is the case in the genetic correlations for Cycles 7-8 (Table 3b). Dominance-to-additive variance ratio was not considered in western white pine since estimates are unavailable from the first generation. It is also assumed that the variance of an individual observation has no maternal, paternal, or reciprocal effects.

Since increasing the number of western white pine tests per cycle is not an option due to limited resources (e.g., high cost of rust screening), efficiencies and precision are determined among mating designs without consideration for increased test sites.

Partial designs, particularly diallels, were marginally less efficient than factorials (Van Buijtenen and Burdon 1990). Half-sib designs are superior to full-sib designs for precision of heritability estimates, and the circular mating design is specifically superior in precision to a half-diallel design across all levels of parents, genetic control and location (Huber et al. 1992). Disconnected mating designs have a major limitation on reliably ranking parents in different breeding groups. It is possible to include standards among breeding groups for comparison purposes, but an additional problems lies in identification and availability of reliable standard(s) (van Buijtenen and Bridgwater 1986). In the case of western white pine, the primary decision is to select between a balanced factorial vs. a circular half-sib mating design.

To meet the goal of producing a base population suitable for successive cycles of selection and mating, the breeder must consider coancestry management, production of sufficient numbers of families and individuals to maintain desired selection differentials, along with estimating any "residual" genetic parameters, not estimated in the first generation program. The circular half-sib mating design best meets the needs of the Western White Pine Tree Improvement Program (Figure 3).

Sublining is used to address coancestry management by horizontally subdividing the large Phase II breeding population into several smaller, closed populations (Figure 2). Matings are made within a subline and not between sublines. The assignment of individuals to a subline is based on common geographic origin, to confine related individuals within a subline. The minimum number of sublines is 16 and may be increased up to 18 sublines. The target number of selections per subline is 20, but should not drop below 15 entries per subline. There are to be four full-sib crosses per entry to adequately assess breeding value or general combining ability, i.e., to increase the probability that any parent will have been mated to at least one average or better parent. With 20 entries and four crosses per entry, there are 40 full-sib crosses within a subline, generating a second generation testing population of size 640-720 full-sib families (16 and 18 sublines, respectively).

When given a choice of performing the controlled crosses in the field or in a breeding house, National Forest personnel and IETIC cooperators with the surviving elite trees preferred that the crossing occur at the Coeur d'Alene Nursery. Costs per cross are higher in the field, require certified tree climbers, and there is increased competition for qualified personnel at that time of year in tree improvement and reforestation. The success of controlled crossing can be improved in an indoor facility using accelerated breeding methods, while reducing the number of years required to complete the planned crosses. As a result, all controlled crosses will be performed within a breeding house at the Coeur d'Alene Nursery.

Deployment Strategy

Deployment, in part, involves the selection of the best individual from each subline for inclusion into a soil-based seed orchard. All seed orchard selections would therefore be unrelated. Previous plans have been to choose those individuals which possess one vertical resistance mechanism in addition

to having a high family ranking based on the four horizontal resistance mechanisms.

There are two seed orchard strategy alternatives for deploying these individuals:

1) Randomly mix the resistance mechanisms throughout an orchard using the top individual in each subline and their ramets (clones) or

2) Block the seed orchard by the four vertical resistance mechanisms using the top individual in each subline and their ramets.

Evaluation of seed orchard alternatives. As with the Phase II first generation program, alternative (2) will be given priority consideration to strive for 100% resistance. After the first generation of improvement and nature of developing disease resistance in western white pine, i.e., selecting families for horizontal resistance and individuals within-families for vertical resistance, forward selection remains the only viable selection method for establishing production seed orchards. For additional discussion see the sub-heading on "Seed Orchard Strategy" and "Evaluation of Alternatives" in the Phase II first generation section of this plan.

Establishment of second generation seed orchards. Superior individuals from the second generation breeding program will replace existing first generation seed orchards, but timing will be of importance to ensure a constant seed source supply to meet individual cooperator needs. For the Forest Service, the seed orchards at Couer d'Alene Nursery, Lone Mountain, and Grouse Creek Tree Improvement Area would not be "plowed under" until the second generation seed orchards have achieved reproductive maturity and cone collections are meeting regional seed needs.

A summary of the time table for developing the second generation breeding population and initiation of operational cone collections from the second generation seed orchards is outlined in Table 4. Reducing the time required to complete the second generation program are: (1) cooperator's ability to collect scion and pollen for the indoor breeding house at the Coeur d'Alene Nursery, and (2) completing all full-sib crosses. If these limiting factors can be overcome, it is possible to shorten the second generation interval from 34 to 21 years, thereby increasing gains per year.

ADVANCED GENERATIONS AND BEYOND

Establishment of future breeding populations will now be limited to forward selections. The time interval for initiating the third generation program will be longer since breeding will occur on selected, surviving seedlings from the rust screening trials. The attractiveness of horizontally subdividing the breeding population into sublines is that at any time in the future if objectives and direction change, all 16-18 sublines can be collapsed into one large main-line population, if appropriate.

Inclusion of new genetic material, e.g. identification of new plus trees, may be considered in future generations to broaden the genetic base, recognizing the trade off between increasing the base population, while diluting the gains in previous generations (new material is untested and has not been bred or selected for disease resistance).

Future Direction and Information Needs

* Re-evaluate efficacy of Phase 2 families in Cycles 1-6 field tests using a rust index score, for inclusion into the breeding population.

* Evaluate all Phase II first generation families for increased incidence of a particular vertical resistance type for potential development of three to four elite populations. These elite populations would be in addition to the 16 sublines, to further increase our short-term gains by breeding for four horizontal and one vertical resistance mechanism.

* Need to identify the presence or absence of biologically significant specific combining ability effects utilizing the full-sib families.

* Need of additional genetic tests among second generation full-sib families to further study the presence/absence of genotype-by-environment interations and the stability of the existing breeding zone boundary (Figure 1).

* Possible further consideration of the use of complementary mating and test designs to increase gains within the context of the existing artificial inoculation and rust screening process.

* Possible consideration of utilizing molecular genetics technology for disease resistance screening.

Table 1. Standardized rust screening data collected by inspection periods 1-4.

	Rust Inspections			
	1st	2nd	3rd	4th
Variables	JUN 92	SEP 92	SEP 93	SEP 94
Health (Dead/Alive)	X	X	X	X
Mechanical Damage (Presence/Absence)	X	X	X	X
Needle Spots (Presence/Absence)	X	X		
Total Tree Spot #	X			
Total Tree Yellow Spot #	X			
Total Tree Red Spot #	X			
# Yellow Needle Spots on Top Fasicle	X			
# Red Needle Spots on Top Fasicle	X			
Top Fasicle Length (mm)	X			
Canker(s) (Presence/Absence)		X	X	X
Bark Reactions (Presence/Absence)		X	X	X
Four-year height (cm)				X

Table 2. Rust resistance traits in western white pine.

Horizontal Resistance Mechanisms (among families)		Vertical Resistance Mechanisms (by individual)	
Needle lesion frequency	(SPM)	No Spots	(NT)
Early stem symptoms	(ESS)	Needle Shed	(NS)
Canker Alive (Tolerance)	(CA)	Fungicidal Short Shoot	(SS)
Adjusted Bark Reaction	(BRCA)	Bark Reaction	(BR)

Based on plot mean (family) data by replication:

Needle Lesion Frequency (spots per meter) = (Number of Yellow Spots on Top Fasicle / Fasicle Length) * 1000;

Early Stem Symptoms = Number of stem sypmtoms (Cankers and Bark Reactions) in Second Inspection/ Number of stem symptoms (Cankers and Bark Reactions) in Fourth Inspection.

Canker Alive (tolerance) = 1 if canker present in fourth inspection and tree is still alive; else tolerance=0.

Adjusted Bark Reaction = Total number of bark reactions in a family plot during inspections 2-4 / (Total number of seedlings in family plot - no spot - needle shed - fungicidal short shoot trees).

Based on individual-tree data:

No Spots = no spots in **both** the first and second rust inspections and no bark reactions in the fourth inspection.

Needle Shed = seedling had spots in first inspection, all spots were absent in second inspection and no bark reactions in the fourth inspection.

Fungicidal Short Shoot = No cankers or bark reactions in the fourth inspection and spots are present in the second inspection.

Bark Reaction = presence of any bark reactions in the second through fourth inspections.

Table 3. Genetic Parameter Estimates for Cycles 7-8, Horizontal and Vertical Resistance Traits.

(a) Variance Components:

White Pine Cycle 7

Trait	VA	VP	h2(F)	%Gain
ESS	0.10	0.04	0.65	-12.0
SPM	93.76	91.54	0.26	- 2.5
BRCA	0.01	0.003	0.65	5.7
CA	0.05	0.026	0.49	8.8

White Pine Cycle 8

Trait	VA	VP	h2(F)	%Gain
ESS	0.06	0.06	0.54	-13.1
SPM	776.70	3662.74	0.19	-12.2
BRCA	0.01	0.01	0.62	6.9
CA	0.02	0.02	0.61	6.8
HT4	124.57	105.57	0.61	3.9

VA=additive genetic variance, VP=total phenotypic variance, h2(F)=family heritability, and Gain=percent expected gains. ESS=early stem symptoms, SPM=spots per meter (needle lesion frequency), BRCA=adjusted bark reactions, CA=canker alive, and HT4=nursery bed height, four years from seed.

Table 3 (continued). Genetic Parameter Estimates for Cycles 7-8, Horizontal and Vertical Resistance Traits.

(b) Phenotypic Correlations Below Diagonal (rP), Genetic Correlations Above Diagonal (rG):

White Pine Cycle 7

		Horizontal Traits				Vertical Traits			
	rP\rG	ESS	SPM	BRCA	CA	NT	NS	SS	TBR
1	ESS		+0.36	-0.82	-0.41	-	-	-0.90	+1.17
2	SPM	+0.37		-0.17	+0.14	-	-	-0.14	-0.44
3	BRCA	-0.46	-0.14		+0.42	-	-	+0.92	+0.92
4	CA	-0.49	-0.41	+0.24		-	-	+0.46	+0.75
5	NT	-	-	-	-		-	-	-
6	NS	-	-	-	-	-		-	-
7	SS	-0.46	-0.29	+0.37	+0.44	-	-		+0.75
8	TBR	-0.22	-0.13	+0.42	+0.13	-	-	-0.00	

White Pine Cycle 8

		Horizontal Traits				Vertical Traits				
	rP\rG	ESS	SPM	BRCA	CA	NT	NS	SS	BR	HT4
1	ESS		+0.61	-0.92	-0.91	-	-0.87	-0.59	-0.44	-0.24
2	SPM	+0.31		-0.44	-0.35	-	-0.28	-0.32	+0.23	-0.61
3	BRCA	-0.44	-0.17		+0.42	-	+0.99	+0.88	+0.61	+0.18
4	CA	-0.51	-0.22	+0.33		-	+0.48	+0.47	+0.75	+0.45
5	NT	-0.11	-0.18	+0.03	+0.08		-	-	-	-
6	NS	-0.24	-0.11	+0.20	+0.18	+0.08		+1.30	+0.40	+0.59
7	SS	-0.37	-0.28	+0.37	+0.30	+0.07	+0.01		-0.01	+0.02
8	BR	-0.22	-0.24	+0.36	+0.38	+0.12	-0.01	+0.07		+0.28
9	HT4	-0.39	-0.31	+0.18	+0.24	+0.10	+0.18	+0.27	+0.13	-

NT=no spot trees, NS=needle shed, SS=short shoot, BR=bark reaction, TBR=total bark reaction (only used in Cycle 7 analyses); others defined above.

Blanks occur where data are lacking, variability in inoculation procedures do not afford construction of some variables, and/or the problems inherent in deriving genetic correlations between quantitative and qualitative data.

The only unfavorable genetic correlation in Cycle 7 is between spots per meter (SPM) and tolerance (CA canker alive) at 0.14. Early stem symptoms (ESS) "appears" to have an unfavorable genetic correlation with total bark reactions (TBR), but is not biologically significant since TBR is a composite trait made up of components that are selected for in "opposite" directions, and is further disregarded in this cycle of 200 open-pollinated families.

Table 4. Time table for second generation program.

Year	Activity
1993	Initiation of second generation breeding population development.
1995	Begin controlled pollinations within developing sublines at Coeur d'Alene Nursery.
2003	First generation Phase II testing program completed.
2004	Complete grafting of second generation breeding population among sublines at Coeur d'Alene Nursery.
2007	Final full-sib crosses made within sublines.
2008	First nursery rust screening trial for second generation program.
2013	Begin establishment of second generation seed orchards.
2015	Complete second generation testing program.
2027	Second generation seed orchards begin to reach reproductive maturity.

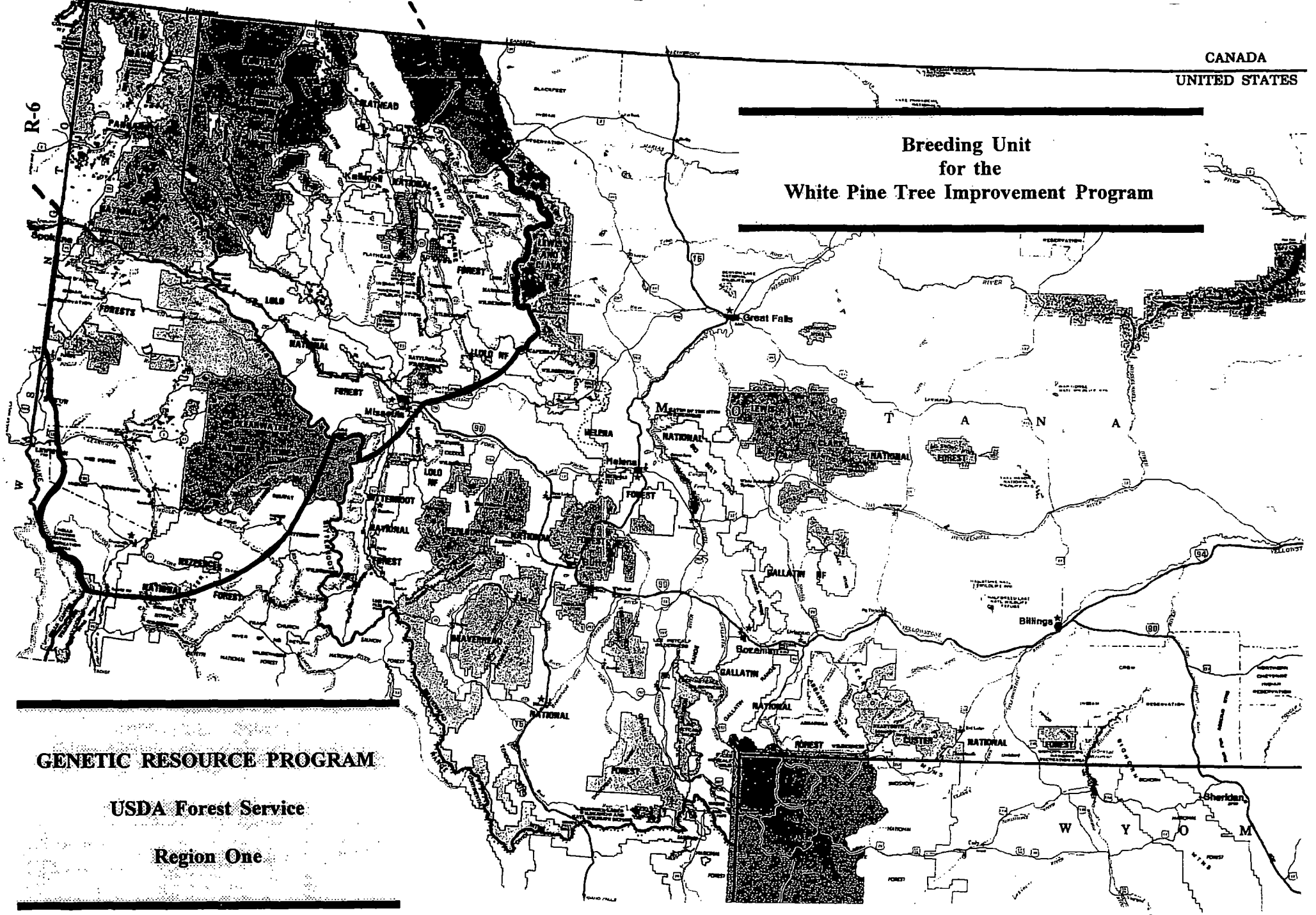


Figure 1. Breeding unit designation for Western white pine genetic resource program.

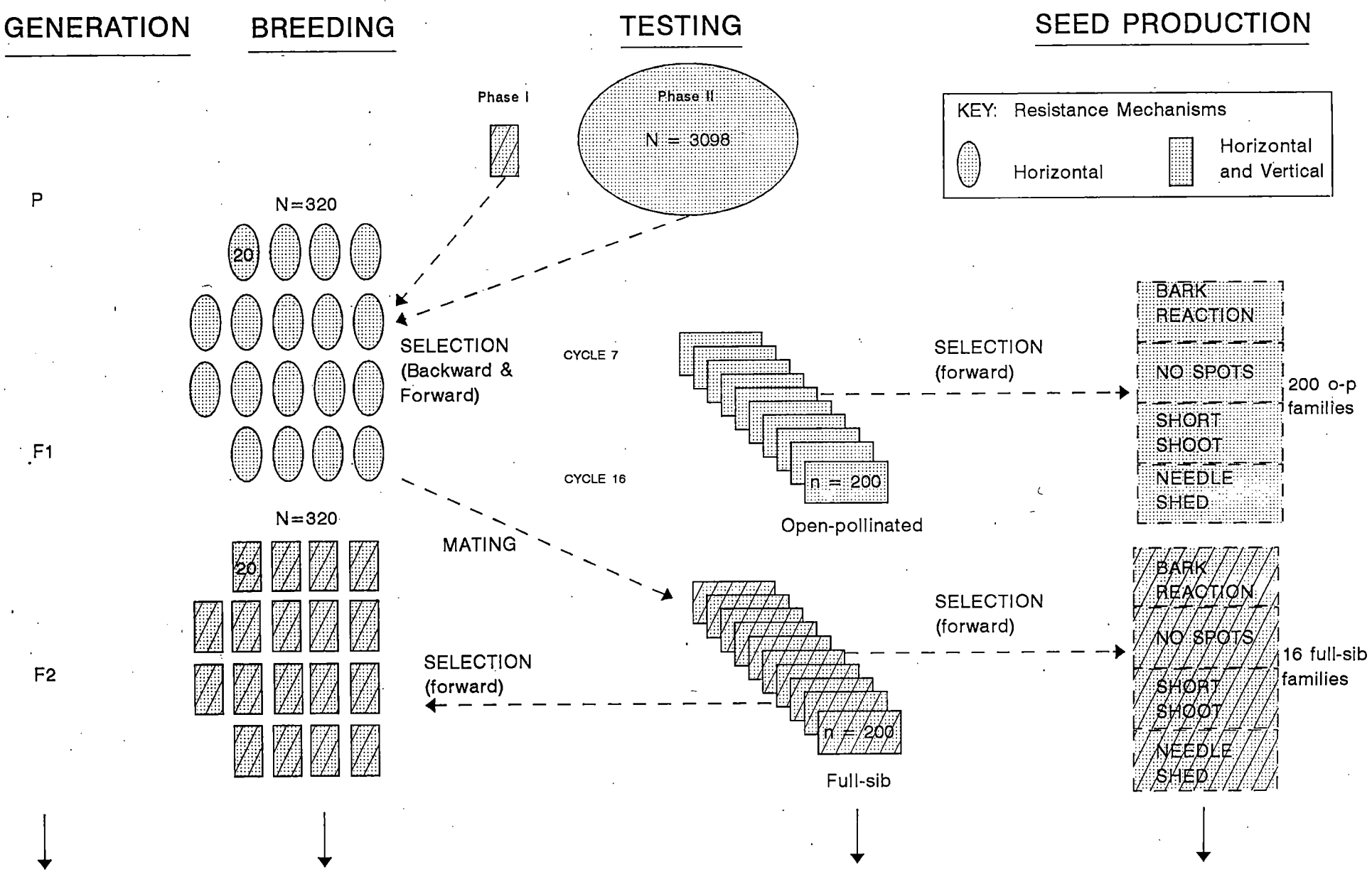


Figure 2. Western white pine recurrent selection program.

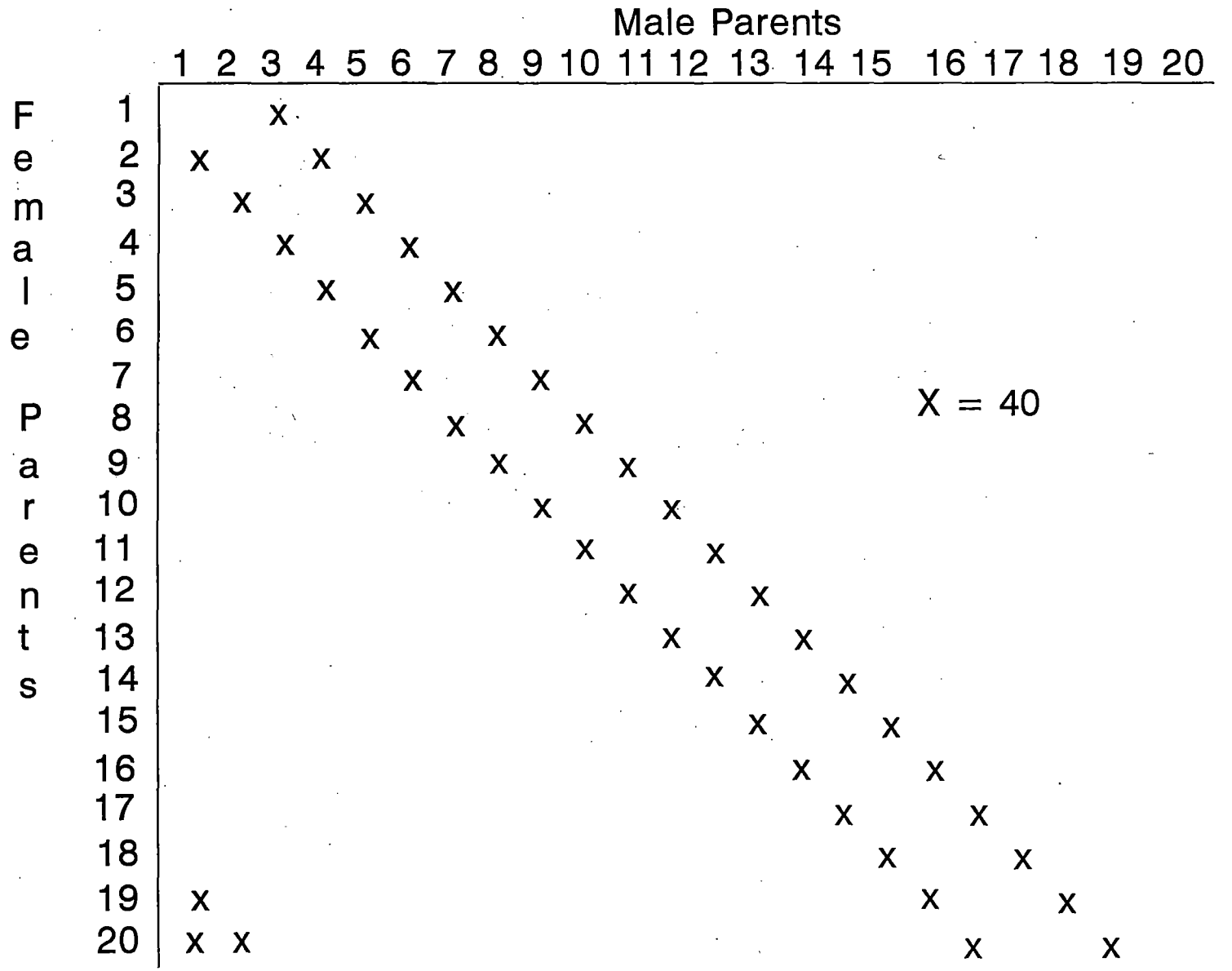


Figure 3. Circular half-sib mating design within a second-generation subline.

Appendix A. Phase II Candidate (Plus) Tree Selection Procedures.

A. Stand Selection Criteria. The following criteria were used to identify stands where candidate trees were to be located:

1. with a high proportion of living or dead white pine.

2. in easily accessible by road or trail.

3. in uniformly and heavily infected with blister rust (10 or more cankers per tree on the average).

4. not less than 25 years old and from 35 to 100 feet tall so that the stand will:

    a. have had 25 years of exposure to blister rust;
    b. be of cone bearing age;
    c. be easily and safely climbable.

5. of moderately open stand density so that the candidate trees will:

    a. be easy to examine from the ground;
    b. have persistent branches to the ground for climbing ease;
    c. have full crowns for better cone bearing potential.

B. Tree Selection Criteria. Candidate trees were selected according to the following criteria:

1. The potential candidate tree, had to be relatively free of blister rust as compared to the infection level in the entire stand. Allowable infection in the candidate tree is summarized in the following table:

Stand Average	Candidate Tree Limit
10 to 20 cankers/tree	no cankers
21 to 40	1 canker
41 to 75	2 cankers
76 to 150	3 cankers
151+	4 or 5 cankers

Even in the most heavily infected stands it was possible to find canker-free trees and seldom were trees selected with more than 1 or 2 cankers.

2. The presence or absence of cankers in a potential candidate tree was determined by examining the tree from the ground and by climbing the tree and examining each individual whorl from bottom to top.

3. The average stand infection level was determined by counting both live and dead cankers on at least three living or dead white pines in the stand. Where rust infection was heavy and mortality was high, allowances were made for the possible presence of difficult to see or undetectable cankers.

4. The potential candidate tree had to have a history of, or the potential for, cone bearing. The presence of current or persistent past cone crops was noted. (A small portion of the original candidate trees have subsequently proven to be sterile.)

5. Candidate trees were to be within five to 10 chains of the nearest road or trail, unless intervening vegetation was sparse enough so that longer lines of sight were possible.

6. Only dominant or co-dominant trees, free of defect or deformity, were selected as candidate trees.

C. Marking and Locating Procedure. The following procedures were used to identify, mark, and locate candidate trees:

1. Candidate trees were identified with a 4-digit number. A series of numbers were assigned to each of the cooperating "white pine" Forests.

2. Each candidate tree was marked with an aluminum number tag, an orange location marker, and orange paint bands above and below the tags. The number of the candidate tree was also recorded on the orange location marker.

3. A white muslin cloth bag was tied over a branch in the top of the tree and orange flagging was tied to several branches at the base of the tree to aid in relocating the tree.

4. The distance and bearing to the candidate tree, from two widely separated departure trees on opposite sides of the nearest access road, were determined and each departure tree was tagged with an orange location marker. The marker was inscribed with the candidate tree number and the distance and bearing to the candidate tree. The departure trees were also painted with an orange paint band. One location marker could be used to locate up to three different candidate trees in stands where numerous candidates had been located.

5. The candidate tree markers have been maintained or remonumented as needed. At the present time, there are no plans to locate additional new candidate trees.

D. Cone Collection and Handling Procedure:

1. Approximately 10 to 20 cones were collected from each candidate tree.

2. The cones were bagged at the beginning of the second growing season to protect them from cone insects and squirrels.

3. Each seed lot is identified by candidate tree number.

E. Extraction and Storage. Cones were shipped to the Coeur d'Alene Nursery for extraction and storage. A maximum of 1,600 seeds are stored for each seed lot. Trees short of seed are recollected as needed.

Appendix B. Inoculation Procedures.

Source Material

The inoculum source comes primarily from an established Ribes garden at Lone Mountain Tree Improvement Area. Clones in the garden have been selected for being good carriers of blister rust.

Timing

Seedling inoculation is scheduled in late August of the second growing season, when teliospore development on the alternate host is at a maximum. Timing of the inoculation is keyed to the development of the telio horns on the underside of the Ribes leaves on the bushes in the Ribes garden at Lone Mountain.

Inoculum Collection

Ribes bushes are inoculated in mid to late June with aeciospores collected from active blister rust cankers. The aeciospore mix is made up of collections from across the range of the Inland white pine breeding unit (Figure 1). The disease is then intensified on the infected Ribes bushes by uredia transfer during late July and early August. Branches from infected plants are used to spread the uredia spores to non-infected material. The ribes garden is irrigated frequently during this period to maintain high relative humidity in the garden structure, which helps the spread of uredia from infected bushes to non-infected ones.

Inoculum is collected from the Ribes garden when telia horns have ample basidiospore production. The timing of the collection is determined by "plating" sampled leaves in agar petri dishes. The leaves are kept in the petri dishes overnight to allow time for spore drop. The dishes are inspected by microscope. A decision is made to collect leaves from the Ribes garden when the average spore drop count has reached 5-10 spores per dish.

Approximately 2400-2600 Ribes leaves are collected for each inoculation run per year. The Ribes garden is equally divided into 12 sections prior to collection. Each section is represented by five clones. The number of leaves per clone per section, is determined by: 1) the rate of infection, and 2) inoculum production present. The goal is to collect at least 200 leaves per section. In good infection years, collections are divided equally among the five clones (approximately 40 leaves per clone). In sections where inoculum is not equal across all clones, 40 leaves are collected from as many of the clones as possible and any shortages are supplemented from collections from the clone "nigram". If available, leaf samples from wild sources are also collected.

Ribes leaves are collected no sooner than 24 hours prior to the inoculation. Harvested leaves are packaged in groups of 40 in plastic sandwich bags. A small amount of water is added to the bottom of each bag to keep the leaves moist and to prevent the telia from drying out. Leaves are stored in camp coolers for transportation from the collection point, and are refrigerated until used.

Inoculation Chamber

An inoculation chamber is created by tightly enclosing a double hooped framehouse with plastic and canvas to maintain optimum humidity and

temperature, and to minimize air movement. Soaker hoses placed on the floor are used to maintain humidity in the inoculation structure as close to 100% as possible. Humidity is maintained by thoroughly wetting down the interior of the chamber from top to bottom for 24 hours prior to inoculation and by operating soaker hoses in the chamber during the inoculation to keep the wood chips on the chamber floor wet. Temperature is maintained at close to 60°F by sprinkling the exterior canvas shell continuously during the inoculation run. Temperature and humidity are monitored by a hygrothermograph placed among the flats of seedlings in the chamber.

Seedling Prep

Families are separated into three replications (some cycles had four replications) prior to the inoculation. Seedlings are randomized within a family prior to being placed in the inoculation chamber. Each family of seedlings are put into three replications of either 36, 30, 24, 18 or 12 seedlings per family per replication. Families with less than 36 total seedlings are not tested. There is some grouping among families, based on total height, to ensure an equal inoculation exposure among short and tall families. Seedlings are also randomized within each rep by placing only 6 seedlings per family per super cell tray. This spreads each family throughout the inoculation chamber and minimizes the chances of a family not being inoculated or poorly inoculated, due to potential "hot spots" in the chamber. Lastly, the seedlings are watered before inoculation.

Inoculum Deployment

Infected Ribes leaves are spread telio side down on chicken wire screens and are suspended 18 inches above the trays of seedlings. The bags of leaves are numbered by clone and section from collections in the Ribes garden. Each bag of leaves is spread randomly across all the screens (approximately 5 leaves per screen @ 40 leaves collected). This procedure ensures that no one area in the inoculation chamber has only one clone contributing spores, due to potential differences in inoculum yields among the five clones. This method also eliminates the need to rotate the screens throughout the chamber. After leaf placement is complete, the screens are covered with thin-gauge clear plastic to prevent leaf movement and to minimize the chances of spores being washed away by water from ceiling drip.

Spore cast is monitored by placing microscope slides coated with Bactar agar at 10 predetermined locations throughout the seedling trays. Approximately eight slides are placed at each monitoring station. The objective is to have enough slides throughout the chamber to be able to monitor spore drop, calculate spore germination and total spore drop, and to allow for adjustments due to any mishaps that can occur during this process. Spore count slides are elevated to tree-top level by placing them on concrete blocks. Once the monitoring slides have been placed at each predetermined location the inoculation chamber is sealed for approximately 6 hours before the monitoring process begins.

Monitoring

Slides are collected at 2-hour intervals during the first few hours of monitoring. The interval at which slides are collected is determined by the rate of spore drop and the change in amount of spores dropped from one 2-hour period to the next. If spore drop is rapid, slides may be taken at one hour intervals following the first two readings. Spore cast is determined by

staining the collected slides with analin blue and counting the basidiospores with a binocular microscope.

Spore cast is measured in spores per square centimeter and is determined by counting the spores in a prescribed number of fields and multiplying by a given conversion factor. Ten fields are counted on each of the 10 slides pulled during a monitoring period. In addition to the count slides, 10 permanent slides are established among the seedlings. These slides are prepared and read in the same manner as the count slides; however, they are put in place at the beginning of the inoculation and are not stained and read until the inoculation is complete. They provide a double check on the total spore cast and are used to determine germination of the spores. Spore germination is determined approximately eight hours after the inoculation has been completed. When the cumulative spore cast reaches 6,000 spores per square centimeter, the inoculation is terminated.

Dismantling

The inoculum is removed immediately upon achieving the desired spore cast. The inoculation chamber is left intact for 48 hours following completion of the inoculation. Misting is discontinued to allow the seedlings to dehumidify gradually and improve the chances of successful infection of the seedlings by the germinating basidiospores. The seedlings are removed from the chamber at the completion of the inoculation and are returned to the breeding house. The trays are arranged to maintain the same replications used during the inoculation. The seedlings are watered and cultured to maintain health and vigor, but no treatments are applied to enhance growth.

Cold Storage and Transplanting

Once the seedlings go dormant, around December, inoculated seedlings are tagged and packed by replication, then placed in cold storage at 28°F, until the following spring. Prior to 1 May, the seedlings are removed from cold storage and are transplanted to seedling beds at the Nursery. Transplanted seedlings are watered, fertilized, and weeded, as necessary for the duration of the rust-resistance testing.

Appendix C. Aeciospore field collection guidelines.

Objectives

Aeciospore collection is something that will be necessary every spring to meet program needs. It has become apparent that some guidelines are necessary to ensure quality collections. In order to ensure that we have adequately covered the racial variation, which occurs in white pine blister rust during our rust testing process, it is imperative that we sample aecia within the same geographic area from which plus trees are selected, and from areas into which seedlings resulting from orchards will ultimately be outplanted.

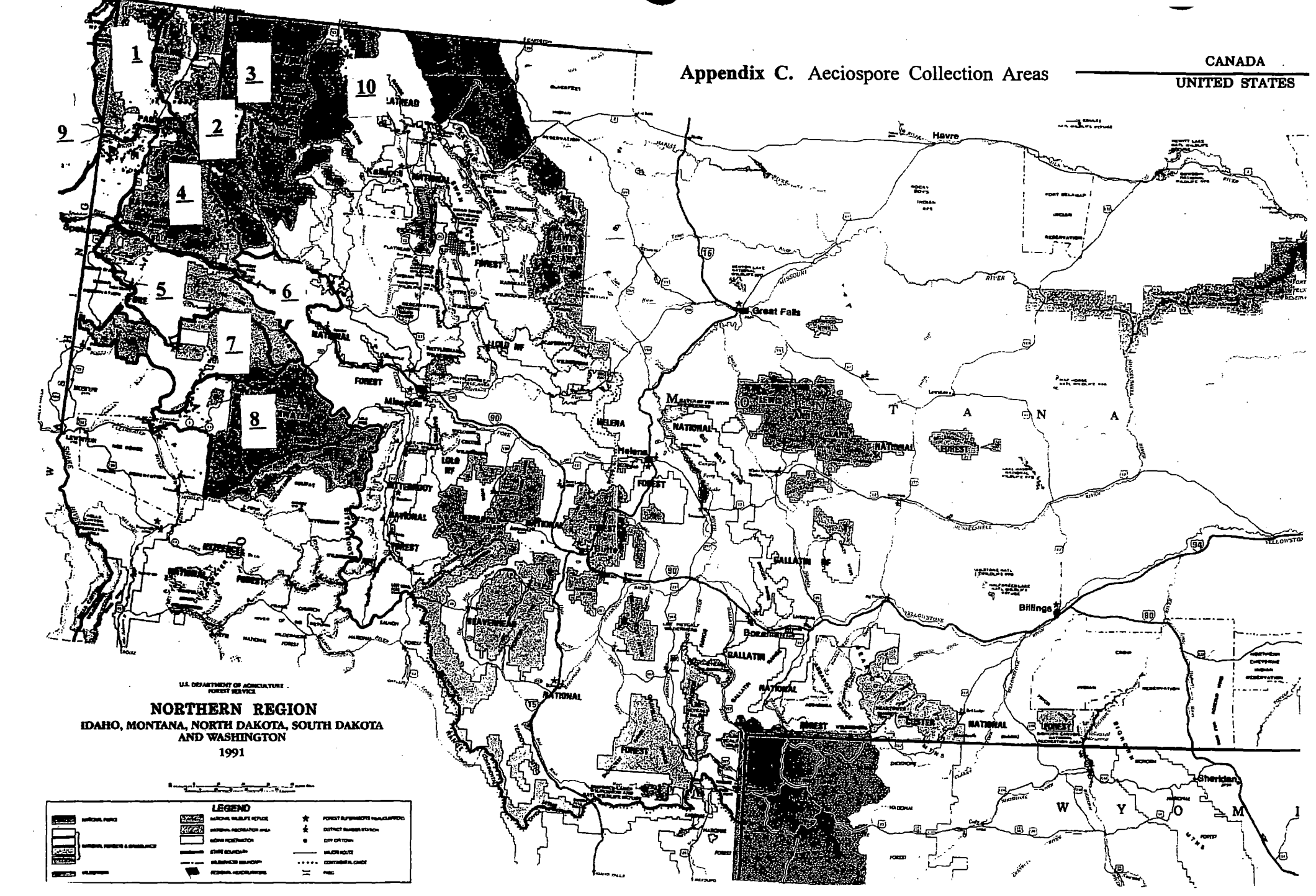
Procedures

The following is a list of general collection techniques and equipment needs. Two collections are needed per field unit per year, in north Idaho and western Montana. A collection consists of aecia from at least three cankers from the same general area. Individual collections should be from different drainages, or at least separated by a distance of one mile. The greater the geographic area sampled, the better. Cankers are most easily found by simply driving the roads in known white pine areas. Regeneration along roadside cuts in the 5-15 year old range are usually a good source. Aecia usually begins to release from early April until June depending upon elevation, aspect, etc. Aecia should not be collected until it has dried sufficiently to release naturally. Spores should be collected only during dry weather.

There are several ways to collect spores, the best being the Cyclone collector which can be obtained by contacting Aram Eramian (DG:R01F04A, phone 208 765-7372 or 765-7392). Another method, to use in a pinch, is to cut limb or bole cankers from smaller trees with a pair of pruners and simply tap the limb over a piece of paper to release spores.

Regardless of method used, a bare minimum collection should be no less than 5 cc's with 10-20 cc's being the target to shoot for. Spores which are shedding naturally still have far too much moisture to be transported in a "sealed" container. It's a good idea to air dry the spores in an open petri dish or equivalent, in a low humidity area, for a few hours before transporting to the nursery. Containers can then be closed by inserting a piece of cotton in the top rather than sealing tightly with a lid or cap. Spores should be kept relatively cool and out of direct sunlight. Petri dishes, cotton, etc. can also be obtained by contacting Dave Brown, Sue Waltz or Aram Eramian at the above listed numbers. Delivery should be made to the Coeur d'Alene Nursery (ATTN:TI) AS SOON AS POSSIBLE AFTER COLLECTION! A completed aeciospore collection form should be mailed with the collections.

Appendix C. Aeciospore Collection Areas



Appendix D. Summary of statistical analyses using SAS for each test cycle.

SAS Program File	Analyses Performed:
*[1]FRQ.SAS	Frequency of observations for survival and terminal damage, univariate statistics for quantitative variables, phenotypic covariance and correlation among variables.
*VAR.SAS	Variance component analysis of quantitative variables, ranking of top 30 families using a selection index, overall test and seed orchard family means, selection sheets for breeding and seed orchard construction.
*INDX.SAS	Selection index coefficients using IML.
*GSTAT.SAS	Estimates of genetic parameters: phenotypic and genetic variances and standard errors at family, individual, and with-in family levels; family, individual, and within-family heritabilities and standard errors.
*GCORR.SAS	Estimates of genetic covariances, correlations, standard errors, and coefficient of genetic prediction.
*SOSEL.SAS	Generates listing of seed orchard selections.

[1] * wild card replaced with species and Cycle number codes, i.e. WP7FRQ.SAS for Cycle 7.

Appendix E. Summary of field test establishment.

Objectives

(1) To evaluate long-term individual and family performance on natural sites. Data from field test will be used to: (2) rogue seed orchards, and (3) provide select material for advanced generation breeding.

Site Selection

A habitat type in the Pachistima union in which white pine would be a desirable or acceptable component. One site is selected per cycle.

Design

The randomized complete block design is initiated in Cycle 8, and replaces the completely randomized design used in cycle 7 (**assumes Scofield, Bertha, Cedar, etc. all had blocking**). Randomized complete block design is to have a minimum of three replications, using single-tree plots. Number of families and individuals will vary depending upon number of trees available with no active cankers, after seed orchard selections have been made. Seedlings (5-2) are planted at a 10' x 10' spacing. Each seedling tag identifies family number, individual number, vertical resistance mechanism, and nursery bed row and column locations to simultaneously track nursery and field performance. Each field test is surrounded by two border rows, which in some cases is comprised of several conifer species.

Site Prep

As specified in the Silvicultural Prescription for the timber harvest activity on the selected site. Site preparation is normally done by dozer scarification or prescribed burn. Additional treatment of ground cover may be required when site preparation precedes plantation establishment by more than one year.

Layout

Laid out prior to planting in a 100-tree grid system with rows and columns following procedures described in the chapter 500 of the Tree Improvement Handbook.

Establishment and Maintenance

Plant as soon as the site in ready the first spring following the fourth rust inspection. Use planting bars, mattocks, augers, or other planting devices, but no dibble planting. Selected seedings are to be lifted and handled like bareroot planting stock.

Portable data recorders are used immediately after planting to record each tree location (replication, row, and column), family identification, resistance mechanism, original nursery location (replication, row, and column) etc., to produce a map of the test site and to aid in data acquisition and for future measurements.

A plantation guide will be developed for each field test, by the plantation manager, in the first growing season. Components of the plantation guide that are the responsibility of the plantation manager: Test Plantation

Information Sheet, Test Plantation Establishment Report (both of these reports are available on the DG from Moscow FSL), daily journal of test establishment conditions (e.g., date, weather conditions, crew members, areas or replications planted, problems encountered, etc.), 8 1/2 x 11" general map identifying test location (xerox area off a Rand McNally road map), 8 1/2 x 11" map identifying the test location by township, range, and section (xerox area off a National Forest map), a schematic of the test location within the section, and a schematic of the test layout showing replication boundaries, with beginning row and column designations, and cardinal orientation. This information should be mailed to Selective Breeding Specialist at USFS Moscow FSL, duplicate copies will be sent to UI, IETIC. In addition, two permanent photo points need to be established. Good quality 35mm color slide film should be used: send one copy to Marc Rust at the University of Idaho and a duplicate set to the Computer Specialist at Moscow Forestry Sciences Lab. Slides of these photo points need to be taken at 5-year intervals.

Components of the plantation guide that are the joint responsibility of data management at USFS Moscow FSL and IETIC: production of a map by family, row, and column, gathering all documents into one guide, with distribution of the guide to interested parties. Over the lifetime of the test, management activities are to be recorded in the same manner as the daily journal for establishment. Updated copies should be sent to Moscow FSL for updating the Plantation Guide at the end of each field season.

Monthly quick checks (approximately six per field season) are performed in each field test per the guidelines in Chapter 500 of the Tree Improvement Handbook.

Measurements and Analysis

Field test measurements will be recorded on electronic field recorders. Identity is verified and survival is measured after the first growing season. Survival after the second, third, and fourth growing seasons, as needed. Survival, rust development, and height growth are measured after the fifth, seventh, tenth, fifteenth, and twentieth growing seasons and periodically thereafter as deemed necessary. Data on damaging agents identified in quick checks are also collected. Contingency measurements are warranted to... Data analyses summarized in Appendix D will be followed; traits of interest will very among cycles depending upon specific site locations.

1. Survival is measured at the end of the first growing season. This is a 100% check and serves as a verification of the computer generated map.

2. Survival checks are made after the second, third and fourth growing seasons only as needed and are by sample only following the procedures used in operational plantations.

3. Rust development and height growth are measured at the end of the fifth, seventh, tenth, fifteenth and twentieth growing seasons and periodically thereafter as long as useful genetic information can be obtained.

4. Opportunistic measurements of damaging agents other than white pine blister rust, that may yield useful genetic information, will be made on a case by case basis.

Appendix F. Seed Orchard Establishment.

Marking

Seedlings in the transplant bed that have been identified as seed orchard trees are marked for lifting in early March.

Lifting

Seedlings are mechanically lifted with as little damage to the root system as possible. Seedlings are handled using standard lift and pack procedures. Seedlings will be root pruned to 18 inches, placed in seedling bags and stored in the tree cooler at 34°F until the seed orchard site opens for planting.

Planting

Planting holes are dug with a 12-inch auger just prior to planting. Seedlings are watered-in after planting. Fertilization is covered in the maintenance section below.

Design

Seed orchards will be planted on a 20 by 20-foot spacing. Seedlings for each resistance mechanism are planted in a group to increase the gain of a particular mechanism.

Maintenance

Transplanted seedlings will be fertilized with B1 or Peters Hi-Phos 15-30-15 fertilizer plus micronutrients at the time of planting and irrigated to prevent fertilizer salt build-up in the root zone. Seed orchard trees will be fertilized in subsequent years with a balanced fertilizer at the rate of 1/4 pound per tree actual nitrogen during the first three years and increasing the rate as trees get larger.

Irrigation will be scheduled as needed to maintain soil moisture for growth. Timing of applications will be based on soil moisture release curves and/or pre-dawn pressure bomb readings.

Cover crops that are non-rhizomatous will be established on the site either prior to or following establishment of the orchard. Cover crop should be maintained to prevent competition with tree seedlings with mowing and herbicides.

Insect monitoring traps will be used for seed and cone insects in the seed orchard and will be coordinated through FPM entomologists.

Appendix G. List of Plantations and Seed Orchards Established.

A. Phase I

1. Moscow Arboretum

Location:	T39N R6W S12 on University of Idaho land just west of the campus about 1/2 mile.
Acres:	28
Planted:	1957-1961, 1964-1967
Stock:	1,376 of the trees are seedlings of western white pine that have survived intense artificial inoculation by blister rust. There are 44 grafts of candidate trees.
Resistance Level:	About 60 percent.

2. Canyon Creek

Location:	T58N R4W S29 within the Lower Canyon Creek drainage at Priest River Experimental Forest.
Acres:	17
Planted:	1971
Stock:	5,500 F_1 and F_2 trees which are survivors of intense artificial inoculation by blister rust.
Resistance Level:	60 percent.

3. Merry Creek

Location:	T43N R2E S17 and 20 in the West Fork of Merry Creek drainage near Clarkia, Idaho.
Acres:	36
Planted:	1970
Stock:	This is a field test for blister rust resistance. It contains nine 1-acre plots of (1) controls,(2) F_1's, (3) B_1's, and (4) F_2's.
Resistance Level:	Controls very low, near 0. About 60 percent for F_1's. About 70 percent for B_1's.About 90 percent for F_2's.

4. Jaype

Location:	T37N R5E S28 across from the Jaype Mill (Potlatch Corporation), 3 miles north of Pierce, Idaho.
Acres:	20
Planted:	1972
Stock:	F_2 western white pine resistance materials. Interplanted with grand fir and Douglas-fir.
Resistance Level:	80-90 percent.

5. Hog Meadows

Location:	T40N R1W S11 east of the town of Deary, Idaho.
Acres:	3
Planted:	1974 with 6-0 trees.
Stock:	2,000 F_2 trees that are survivors of intense artificial inoculation by blister rust.
Resistance Level:	60 percent.

6. Priest River Vigor Quality Plot

Location:	T58N R4W S26 within the Benton Creek drainage at Priest River Experimental Forest.
Acres:	5
Planted:	1955, 1956, and 1957.
Stock:	1,200 trees F_1 stock of candidate and rust resistant. The seedlings were not inoculated but there has been about 38 percent mortality so the resistance genes are at a higher frequency now than before.
Resistance Level:	40 percent.

7. Deception Creek Vigor Quality Plot

Location:	T51N R1W S32 in the Snyder Creek drainage at Deception Creek Experimental Forest.
Acres:	5
Planted:	1955, 1956, and 1957.
Stock:	1,500 trees F_1 progeny of candidate rust resistant trees. The seedlings were not inoculated but there has been some mortality so the resistance genes are at a higher frequency now than before.
Resistance Level:	40 percent.

8. Gletty Creek

Location:	T32N R42E S11 and 12 in the Gletty drainage west of Usk, Washington. This plantation is now on Region 6 lands.
Acres:	6
Planted:	1971.
Stock:	This is a field test for blister rust resistance. It contains nine 1-acre plots of (1) controls, (2) F_1's, (3) B_1's, and (4) F_2's.
Resistance Level:	Controls very low, near 0. About 60 percent for F_1's. About 70 percent for B_1's. About 90 percent for F_2's.

9. Emerald Creek

Location:	T42N R1E S4 in the Emerald Creek drainage near Fernwood, Idaho.
Acres:	5
Planted:	1955, 1956, and 1957.
Stock:	461 trees F_1 progeny of candidate rust resistant trees. The seedlings were not inoculated but there has been some mortality so the resistance genes are at a higher frequency now than before.
Resistance Level:	40 percent.

10. Elk River Progeny Test

Location:	T40N R2E S11 near Elk River, Idaho.
Acres:	1/4
Planted:	1953, 1954, 1955, and 1956.
Stock:	About 500 trees remain; F_1 seedlings from many phenotypically resistant candidate trees.
Resistance Level:	60 percent.

11. Fernwood Progeny Test

Location:	T43N R1W S1 near Fernwood, Idaho.
Acres:	1/4
Planted:	1953, 1954, 1955, and 1956.
Stock:	About 500 trees remain; F_1 seedlings from many phenotypically resistant candidate trees.
Resistance Level:	60 percent.

12. Coeur d'Alene Low Elevation Seed Orchard

Location:	T50N R4W S3 at the Coeur d'Alene Nursery.
Acres:	14
Planted:	1973
Stock:	1,400 F_1 seedlings from crosses between 12 intensively selected Phase I candidate trees.
Resistance Level:	65 percent.

13. Lone Mountain Mid-Elevation Seed Orchard

Location:	T53N R4W S27 near Spirit Lake, Washington.
Acres:	20
Planted:	1973
Stock:	2,100 F_1 seedlings from crosses between 12 intensively selected Phase I candidate trees.
Resistance Level:	65 percent.

14. Lone Mountain High Elevation Seed Orchard

Location:	T53N R4W S27 near Spirit Lake, Washington.
Acres:	7
Planted:	1973
Stock:	750 F_1 seedlings from crosses between 12 intensively selected Phase I candidate trees.
Resistance Level:	65 percent.

15. Sandpoint Seed Orchard

Location:	T57N R2W S21 near Sandpoint, Idaho.
Acres:	17
Planted:	1960
Stock:	About 1,000 grafts of scionwood from 13 proven candidate trees.
Resistance Level:	35 percent.

B. Phase II

1. Red Star Test Plantation

Location:	T35N R6E S8 on the Pierce District.
Acres:	12
Planted:	1978
Stock:	Open pollinated seedlings from 650 test Cycle I families.
Resistance Level:	20 to 50 percent.

2. Scofield Test Plantation

Location:	T38N R3E S6 on Potlatch Corporation ground near Headquarters.
Acres:	12
Planted:	1978
Stock:	Open pollinated seedlings from 650 test Cycle I families.
Resistance Level:	20 to 50 percent.

3. Cedar Creek Test Plantation

Location:	T42N R5E S28 on Potlatch Corporation land near Elk River.
Acres:	6
Planted:	1979
Stock:	Open pollinated seedlings from 250 test Cycle II families.
Resistance Level:	20 to 50 percent.

4. Bertha Creek Test Plantation

Location:	T39N R6E S6 on the Canyon Ranger District near Headquarters.
Acres:	6
Planted:	1979
Stock:	Open pollinated seedlings from 250 test Cycle II families.
Resistance Level:	20 to 50 percent.

5. Paradise Valley Test Plantation

Location:	T61N R1E S16 on State land near Bonners Ferry, Idaho.
Acres:	12
Planted:	1981
Stock:	Open pollinated seedlings from 700 test Cycle III families.
Resistance Level:	20 to 50 percent.

6. Tired Wolf Test Plantation

Location:	T39N R1E S11 on Potlatch Corporation land near Elk River, Idaho.
Acres:	12
Planted:	1981
Stock:	Open pollinated seedlings from 700 test Cycle III families.
Resistance Level:	20 to 50 percent.

7. Mica Creek Test Plantation

Location:	T45N R3E S9 on Plum Creek land near Avery, Idaho.
Acres:	8
Planted:	1983
Stock:	Open pollinated seedlings from 200 test Cycle II families.
Resistance Level:	20 to 50 percent.

8. Quartz Creek Test Plantation

Location:	T32N R32W S35 on the Libby Ranger District near Libby, Montana.
Acres:	16
Planted:	1983
Stock:	Open pollinated seedlings from 260 test Cycle IV families.
Resistance Level:	20 to 50 percent.

9. Eagle Creek (West Eagle) Performance Test

Location:	T50N R4E S25 on the Wallace Ranger District near Murray, Idaho.
Acres:	1.5
Planted:	1987
Stock:	1275 trees selected from the Cycle IV rust test.
Resistance Level:	50 percent.

10. Deep Creek Performance Test

Location:	T29N R31W S20 on the Libby Ranger District near Libby, Montana.
Acres:	2.5
Planted:	1989
Stock:	1253 trees selected from the Cycle V rust test.
Resistance Level:	50 percent.

11. Oviatt Creek Performance Test

Location:	T39N R1E S12 on the Palouse Ranger District near Elk River, Idaho.
Acres:	1
Planted:	1991
Stock:	800 trees selected from the Cycle VI rust test.
Resistance Level:	50 percent

12. Willow Creek Performance Test

Location:	T43N R3W S42 on the St. Maries District near Emida, Idaho (North/South Ski Bowl).
Acres:	3
Planted:	1993
Stock:	1,502 trees selected from the Cycle VII rust test.
Resistance Level:	50 percent

13. Outhouse Performance Test

Location:	T45N R7E S32 on the Avery District near Hardpan Point
Acres:	10
Planted:	1995
Stock:	selected individuals from the Cycle 8 rust test.
Resistance Level:	50 percent.

14. Lone Mountain Seed Orchard

Location:	T53N R4W S27 near Spirit Lake, Washington.
Acres:	4
Planted:	1982
Stock:	400 selected individuals from the Cycle I and II rust tests.
Resistance Level:	50 percent.

15. Bovill Seed Orchard

Location:	On State land near Bovill.
Acres:	10
Planted:	1982
Stock:	1,400 selected individuals from the Cycle I and Cycle II rust tests.
Resistance Level:	50 percent.

16. Worley Seed Orchard

Location:	On Coeur d'Alene Tribe land.
Acres:	4
Planted:	1982
Stock:	350 selected individuals from the Cycle I and Cycle II rust tests.
Resistance Level:	50 percent

17. Wandless Creek Seed Orchard

Location:	On Diamond International land near Coeur d'Alene.
Acres:	4
Planted:	1982
Stock:	350 selected individuals from the Cycle I and Cycle II rust tests.
Resistance Level:	50 percent.

18. Bakkar Ranch Seed Orchard

Location:	On St. Regis land near Libby, Montana.
Acres:	4
Planted:	1982
Stock:	400 selected individuals from the Cycle I and Cycle II rust tests.
Resistance Level:	50 percent.

19. Cocolalla Outplanting

Location:	On Port Blakely land near Cocolalla.
Acres:	10
Planted:	1982
Stock:	A broad sample of individuals from the Cycle I and Cycle II rust tests.
Resistance Level:	20 to 50 percent.

20. Grouse Creek Seed Orchard

Location:	On the Sandpoint Ranger District near Sandpoint.
Acres:	25
Planted:	1987+
Stock:	Selected trees from the Phase II rust tests and Phase II field tests.
Resistance Level:	60 percent.

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WESTERN WHITE PINE RESISTANCE TRAITS

HORIZONTAL RESISTANCE TYPES	PROGRAM EMPHASIS Phase I	Phase II
Family Selection		
Spot frequency/meter (needle lesion frequency)		X
Early stem symptoms		X
Bark reaction		X
Canker alive (tolerance to infection)		X
VERTICAL RESISTANCE TYPES		
Individual-tree Selection		
No Spots		X
Needle Shed	X	X
Short Shoot	X	X
HORIZONTAL RESISTANCE TYPES		
Individual-tree Selection		
Bark Reaction	X	X

Definitions:

Immunity: complete absence of rust infection (e.g., no branch flags, no cankers) and no mortality.

Resistance: ability to tolerate rust infection, no mortality.

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